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DYNAMICS OF THE SLOVAK CONSUMER BEHAVIOUR IN THE CONTEXT OF ETHNOCENTRISM: MANAGERIAL IMPLICATIONS

SUMMARY

Every company tries to sell its production on the market and has to face the activities of competitors. Various concepts are used to succeed. One of them is the concept of ethnocentrism. Here, it is assumed that the consumer will prefer local production for various reasons. Various studies are devoted to creating and cultivating the consumer's relationship with local products, on which we base this article. The article presents findings from two representative surveys conducted in 2013 and 2020. The respondents were Slovak consumers and the sample was selected using stratified deliberate sampling. There were 1,030 respondents in 2013 and 1,000 in 2020. Respondents were asked the same questions, which guaranteed the comparability of results. The dynamics of consumer behaviour is measured by comparing selected demographic variables of respondents for individual years of research on the topic of consumer behaviour. We focused on three key areas: willingness to pay more for Slovak products, importance to buy Slovak products and whether the Slovak consumer is aware of support Slovak economy, when buying Slovak products.

Using the chi-square test, the relationships between demographic variables and consumer behaviour were determined. Subsequently, consumer segmentation was developed using cluster analysis in the range of tested variables for 2020. Four segments were identified and described in detail. The common denominator of the findings is the fact that Slovak consumers are generally not willing to pay extra for a local product, but a significant majority are aware that by supporting Slovak products they support the national economy. About half of the respondents consider it is important to behave ethnocentrically in their shopping behaviour. At the end of the article, the managerial implications for customer relationship management from the perspective of the government, retailers, customer associations and businesses are formulated.

Keywords: Consumer behaviour, Ethnocentrism, Marketing management, Willingness to pay, Local production.

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INTRODUCTION

Katt and Meixner (2020) examined in their study the drivers that influence consumer willingness to pay for food, in their cases more specific organic food. From the result there can be seen that the drivers in the cluster analyses for willingness to pay are categorized into three categories related to: consumer (demography – gender, age, income, education, household, family size; values and attitudes; consumer behaviour), product (product attributes – price, quality, locality; product signaling; and product-consumer relationship) and purchasing venue-related factors (type of store). With the most positive impact on willingness to pay are linked with the consumer values and attitudes, quality, locality and labelling of the food products and shopping environment. Another study conducted by Zander and Feucht (2018) shows that additional willingness to pay in the case for European seafood was the highest for: organic and sustainable production, with higher animal welfare, local production, without discards and produced in Europe. According to the results of a survey by Kádeková *et al*. (2017) Slovak customers tend to by organic food from domestic producers and see this as support for the local economy, but the price influences the decision. The price impacts the purchase, but the Slovak consumers are willing to buy domestic dairy products the most (Ubrežiová *et al*., 2019). Géci *et al*. (2020) suggest that Slovak consumers are aware of the quality of purchased goods and if they fit their lifestyle.

Findings by Steenkamp (2019) of ethnocentrism show that is affecting the consumers' attitude towards global consumer culture, those customers are more open to experience with a negative attitude to advertising. Consumers with a high attitude toward local consumer culture are more agreeable and are more brands loyal and are more health consciousness and this type of consumer is much more dispersed among sociodemographic groups in society than the other type.

Customers concerns about the environment, health awareness and concerns about the local economy are strong predictors of attitudes towards local foods, which have been found to have a significant effect on the intention to purchase local food (Kumar and Smith, 2018). Holotová *et al*. (2020) findings suggest that Slovak consumers are eco-aware by purchasing the products.

Customers purchase local food more frequently with the link to the local support rather than to product quality and with this behaviour their support local worker and the local economy; this behaviour can be also linked to the local identity shared with the product (Memery *et al*., 2015). Intentions to buy local food are affected by perceived behavioural control and moral norms rather than by attitudes or social factors (Peral-Peral *et al.*, 2022). The local identity of a consumer is a significant predictor local food preference of the consumer (Zhang *et al*., 2022). Taborecka-Petrovicova and Gibalova (2014) stated that Slovak consumers are showing average ethnocentric tendencies and the tendency to domestic or foreign products is ambivalent. The preference towards Slovak products is stronger amongst older consumers and women prefer more than men Slovak brands (Vilčeková and Sabo, 2014). According to findings by Štarchoň

and Weberová (2016) there is no direct link between gender and preference to Slovak brands. Saffu *et al*. (2010) survey shows that for Slovak the local made products are seen as having a better quality but reconsider the price when purchasing. More recent studies by Čvirik (2018, 2019) are dealing with ethnocentrism in Slovakia from a generational and cultural point of view. The younger generation have a lower tendency towards ethnocentrism and Slovakia compared to Spain, Hungary and Italy has a below-average ethnocentrism score. Horváth *et al.* (2021) findings show that online purchase differs amongst the generations.

MATERIAL AND METHODS

Analysis of the theoretical basis of the available knowledge is the basis of this article. The concept of ethnocentrism is currently being explored from various perspectives on consumer behaviour. Several studies are currently published focusing on consumer behaviour in the context of sustainable production, not only of food but also of a textile and a wide range of consumer goods.

Data from two representative surveys, which were conducted on a sample of Slovak consumers, were used to determine consumer behaviour. Sampling reflected proportional stratified selection of respondents based on regional coverage, size of residence/city, age, gender, education and income distribution of subgroups relevant to the republic population.

Conditions of conducted researches are indicated in Table 1.

Table 1: Conditions of conducted researches in 2013 and 2020

	Research 2013	Research 2020
Sample size	1067	1000
Date of research	January/April	December
Likert scales Number	27	30
Demographic questions	5	8
Open questions	3	0
Research conducted by	Department of Marketing, Comenius University, Bratislava	Department of Marketing, Comenius University, Bratislava

Source: authors

The data were statistically evaluated in software IBM SPSS, which allows preparing graphical and numerical outputs. Results of the analysis presented in the table 2 were calculated in IBM SPSS. As the statistical method was chosen Chi-Square Test which allowed testing of factors: gender, age, education and size of residence on three selected statements. Those statements were tested in Likert scale, with 5 stages: absolutely agree, agree, do not know, disagree, and absolutely disagree. The exact p-Value is compared to the critical value of 5% and then the null hypothesis H0 on independence of statements and factor can be rejected or not rejected. The equations for calculating Chi-Square Test of Independence are presented below:

$$\chi^2 = \sum_{i=1}^{R}\sum_{j=1}^{C}\frac{(o_{ij}-e_{ij})^2}{e_{ij}} \tag{1}$$

$$e_{ij} = \frac{\text{row } i \text{ total} * \text{col } j \text{ total}}{\text{grand total}} \tag{2}$$

where o_{ij} is the observed cell count in the i^{th} row and j^{th} column of the table and e_{ij} is the expected cell count in the i^{th} row and j^{th} column of the table. Then calculated X^2 value is compared to the critical value from the X^2 distribution table with degrees of freedom df = (R – 1)(C – 1) and chosen confidence level. If the Calculated X^2 value > critical X^2 value, than could the null hypothesis be rejected.

The measured results in the Chi-Square Test and the comparison of 2013 and 2020 were the starting point for the processing of cluster analysis in the next step. IBM SPSS Software was used for the calculation. Due to the amount of processed data and the idea of the number of segments, a non-hierarchical type of cluster analysis was chosen: K-Means clustering algorithm. Given a set of observations, where each observation is a d-dimensional real vector, k-means clustering aims to partition the n observations into k sets S = {S1, S2, … , Sk} so as to minimize the within-cluster sum of squares. That aims to partition n observations into k clusters in which each observation belongs to the cluster with the nearest mean serving as a prototype of the cluster. What is expected is to minimize the within-cluster sum of squares. The equations for calculating K-Means clustering algorithm are presented below:

$$\arg\min_{\mathbf{S}} \sum_{i=1}^{k}\sum_{\mathbf{x}\in S_i}\|\mathbf{x}-\boldsymbol{\mu}_i\|^2 = \arg\min_{\mathbf{S}} \sum_{i=1}^{k}|S_i|\,\mathrm{Var}\,S_i \tag{3}$$

where μ_i is the mean of points in S_i. This is equivalent to minimizing the pairwise squared deviations of points in the same cluster:

$$\arg\min_{\mathbf{S}} \sum_{i=1}^{k}\frac{1}{|S_i|}\sum_{\mathbf{x},\mathbf{y}\in S_i}\|\mathbf{x}-\mathbf{y}\|^2 \tag{4}$$

Created clusters represent groups of individuals with the same or extremely similar consumer behaviour. This application allows us to apply cluster analysis in marketing segmentation. Created segments may be of interest to economic organizations at the market. Presented outputs are in the form offered by the software solution IBM SPSS.

RESULTS

Independence of statements towards consumer characteristics

The consumer behaviour over the period of seven years was compared. The relationship between selected demographic variables and three scale statements was tested:

1. I am willing to pay more for Slovak products.

2. Buying Slovak products is important to me.
3. By purchasing Slovak products, I support our economy.

The measured results are presented in Table 2. There is described the change in the behaviour of the Slovak consumer over the course of seven years. Highlighted values represent a factor-to-statement independence when the null hypothesis H0 of variable independence can be rejected. Therefore, the alternative hypothesis H1 is accepted, which states that there is 95% independence between the examined variables at the confidence level.

Table 2: Independence comparison of demographic characteristics from attitudes in 2013 and 2020 (measured by chi-square test)

Factor/Statement	I am willing to pay more for Slovak products. *Asymp. Sig. [2-sided]*	Buying Slovak products is important to me. *Asymp. Sig. [2-sided]*	By purchasing Slovak products, I support our economy. *Asymp. Sig. [2-sided]*
Gender	0.519 (2013) 0.332 (2020)	0.058 (2013) 0.088 (2020)	0.052 (2013) **0.029 (2020)**
Age	**0.000 (2013)** 0.485 (2020)	**0.000 (2013)** **0.000 (2020)**	**0.000 (2013)** **0.029 (2020)**
Education	**0.000 (2013)** 0.066 (2020)	**0.000 (2013)** **0.011 (2020)**	**0.000 (2013)** **0.000 (2020)**
Income	**0.028 (2013)** **0.006 (2020)**	**0.000 (2013)** 0.061 (2020)	**0.002 (2013)** 0.146 (2020)
Size of residence	0.053 (2013) 0.485 (2020)	0.092 (2013) 0.090 (2020)	0.604 (2013) **0.018 (2020)**

Source: authors

All the tests have shown that gender does not affect consumer behaviour except that „By purchasing Slovak products, I support our economy.“ as at year 2020. It has been shown that women are more aware of the economy support when buying Slovak products. As many as 91% of women said so. In 2013 generally only 36% of population is willing to pay more for Slovak products, but in 2020 it is 62.9% of population. Buying Slovak product is important for both equally man and woman in 2013 (48.8%) but significantly increased as at 2020 (75.1%) for both genders.

Age has been shown to have a significant effect on consumer behaviour during the period under review as a factor influencing consumer behaviour. The change in consumer behaviour was demonstrated only in 2020, when age ceased to affect the willingness to pay more for Slovak products. The willingness to pay extra for Slovak products has grown steadily with age. While 28.6% of respondents in the 18-29 age group were willing to pay extra, in the age group over 60 it was up to 42.5%. It is interesting that in 2020 the willingness to pay extra for Slovak products increased most significantly among the young generation under 29 (61.1%) and approached the average of the Slovak population (63%). The importance of purchasing Slovak products was the lowest

in 2013 in the age category of 14-17 years (14.3%) and grew continuously up to the age category of 50-59 years (69.4%). In 2020, the positive attitude of young people increased significantly to 61.1% and also increased in the category of 50-59 year-old (81.6%).

Education as a factor in consumer behaviour is significantly important. Only in 2020 did the survey show that education does not affect the consumer's willingness to pay extra for Slovak products. The results for 2013 showed that with increasing education, the willingness to pay extra for Slovak products also increases. Participants with basic education were willing to pay extra to a lesser extent (30.3%) compared to university graduates (44.9%). The situation was similar in the 2020 survey: respondents with basic education showed this willingness to a lesser extent (59.8%) than with university education (72.2%), but the distribution of responses did not show a statistically significant difference in behaviour by education. On the contrary, education is an important factor in the context of the importance of purchasing Slovak products. In 2013, this is important for 25% of the population among respondents with basic education, but up to 57.8% for university graduates. In 2020, the importance increased for the population with basic education to 61% and for university graduates to 82.8%. Education has proven to be an important factor in behaviour even in the case of awareness of support for the national economy. In 2013, this is important for 35.5% of respondents with basic education and 70.3% for university graduates. In 2020, this awareness increased significantly among people with basic education (76.8%), but also among university graduates (94.1%).

Income plays an important role in the willingness to pay extra for Slovak products for both research periods. Given the importance of supporting the national economy and the importance of purchasing Slovak products, income is a statistically significant factor only for 2013. In 2020, income is no longer a statistically significant factor. In 2013, respondents in the income group were willing to pay EUR 1661-2320 (45%) and at least EUR 501-660 (29%) for Slovak products, but it cannot be said unequivocally that with higher incomes the willingness to increase at the same time. In 2020, the willingness to pay in the income category EUR 1,201-1500 (76.6%) was the most significant, the lowest in the income group up to EUR 400 (52.9%). The importance of the purchase of Slovak products was the lowest in 2013 in the income category above 2661 EUR (29.6%) and the highest in the group with income 1991-2320 EUR (66.7%). In 2020, the importance of purchasing Slovak products according to age categories was distributed evenly - mostly in the group with an income of 1201-1500 EUR (79.4%) and the least in the group over 2501 EUR (66%). Awareness of the importance of purchasing Slovak products for the state economy in 2013 was the highest in the income group 1661-1990 EUR (75%) and the lowest in the group over 2661 EUR (50%). In the year, the highest value was measured in groups with income over 1501 EUR (92%) and the lowest in the group 801-1200 EUR (86.3%).

The last variable examined was the size of the residence. It was found that the size of the residence does not affect the consumer attitudes examined over the years. An exception is the significant impact of the size of residence on the awareness of the importance of purchasing Slovak products for the economy in 2020. This is mainly realized by respondents living in cities with 20-100 thousand inhabitants (94%) and the least inhabitants in settlements up to 5 thousand inhabitants (85.8%). In 2013, the lowest awareness of economic support was in settlements over 100 thousand inhabitants (53.2%) and the highest in settlements with the population of 10-20 thousand (64.7%). The importance of the purchase of Slovak products in 2013 was among the inhabitants living in settlements with population over 100 thousand the lowest (42%) and in settlements with population of 10-20 thousand the highest (51.3%). In 2020, the most important to buy Slovak products was measured for residents living in settlements of 5-20 thousand inhabitants (82.6%) and the least important in settlements over 100 thousand inhabitants (69.2%). The willingness to pay extra for Slovak products in 2013 was the highest among respondents from the settlements with 5-10 thousand inhabitants (41.1%) and the lowest at settlements with population over 100 thousand (25.5%). In 2020, the willingness was the highest in settlements with 5-20 thousand (65.1%) and the lowest in settlements over 100 thousand (59.2%).

Table 3: Dynamics of changing the attitudes of the Slovak consumers in 2013 - 2020

I am willing to pay more for Slovak products. *Positive attitude [% of population]*	Buying Slovak products is important to me. *Positive attitude [% of population]*	By purchasing Slovak products, I support our economy. *Positive attitude [% of population]*
36.0 (2013) 62.9 (2020)	48.8 (2013) 75.1 (2020)	56.8 (2013) 88.8 (2020)

Source: authors

Segmentation of Slovak population according to attitudes towards Slovak products

The results of the chi-square test revealed to us the potential for relationships between demographic factors and the tendency to agree with the three selected claims. Factors „gender“ and „size of residence“ demonstrated a low intensity of relationship to the statements tested. On the other hand, „education“ and „age“ were confirmed as significant variables in relation to the statements tested. „Income“ is quite a strong factor, but only for 2014, less so for the variables in 2020.

In the next step, the potential of these factors as segmentation variables as at year 2020 were tested. Due to the size of the data set (1000 respondents and 8 variables), was chosen the K-Means Clustering technique. The aim was to identify those segments that can be considered relevant for exposure to marketing

incentives in the form of a marketing mix and customer relationships programs. The test results are represented in Table 4. The final number of segments reached four. Each cluster refers to a collection of data points aggregated together because of certain similarities. If the data are similar in the whole dataset, the similarities will occur also in the characteristics of clusters. Defined four clusters means the number of centroids which are created. Centroid represents the imaginary location or characteristic of the center of each cluster. Every case (respondent) in dataset is allocated to each of the clusters through reducing the in-cluster sum of squares. K-Means algorithm in this way identifies *k* number of centroids and distributes each case of data to the nearest cluster. The idea is to keep the centroids as small as possible.

Table 4: Centroids of clusters identified 2020

Factor	Centroids of Cluster			
	1	2	3	4
Gender	1.63	1.43	1.44	1.48
Age	5.69	3.88	3.58	3.06
How many inhabitants/city/reside the most?	2.41	2.03	2.22	1.57
What is your highest educational attainment?	2.81	2.84	2.72	2.22
What is your household`s approximately net monthly income?	2.7	4.62	7.22	2.36
I am willing to pay more for Slovak products.	2.44	1.81	2.58	2.58
Buying Slovak products is important to me.	1.98	1.67	2.47	2.42
By purchasing Slovak products, I support our economy.	1.55	1.35	1.70	1.97

Source: authors (IBM SPSS software)

The main purpose of cluster analysis is to include in the segmentation process only those variables that have the potential to contribute to the differentiation of the resulting segments. For this purpose, cluster analysis is linked to the analysis of variance. The value *Sig.* indicates the result in the Tab. 5.

Table 5: Analysis of variance for K-Means Cluster Analysis

	Cluster		Error		F	Sig.
	Mean Square	df	Mean Square	df		
Gender	2.193	3	.244	996	8.975	.000
Age	389.753	3	.976	996	399.434	.000
How many inhabitants/city/reside the most?	38.380	3	1.076	996	35.669	.000
What is your highest educational attainment?	24.605	3	.667	996	36.894	.000
What is your household's approximately net monthly income?	997.610	3	.783	996	1273.718	.000
I am willing to pay more for Slovak products.	30.631	3	.906	996	33.807	.000
Buying Slovak products is important to me.	32.493	3	.874	996	37.171	.000
By purchasing Slovak products, I support our economy.	18.826	3	.724	996	25.985	.000

Source: authors (IBM SPSS software)

It is clear from the results that each of the included variables has a high potential to contribute to the heterogeneity of individual segments. The critical variable 0.05 is higher than measured in each of the compared factors and reaches 0.00 for each factor.

The result of the cluster analysis is the division of respondents into 4 segments. Centroids of clusters testify to their characteristics. The main purpose of cluster analysis is to include in the segmentation process only those variables that have the potential to contribute to the differentiation of individual segments. Centroids offer the information about the characteristics of each segment for the monitored variables.

The first segment consists of a slight predominance of women over the age of 50. They live mainly in settlements with 20-100 thousand residents; have a complete secondary education and a relatively low disposable monthly household income of EUR 800-1200. They are not identified with the willingness to pay extra for Slovak products, it is important for them to buy Slovak products and they identify with the claim that by purchasing Slovak products they will support the Slovak economy. Briefly, they can be characterized as older, living in larger cities conscious but unwilling to pay extra from their low income.

The second segment consists of both genders with a slight predominance of men. It is an age category of 40-49 years living in smaller cities with 5-20 thousand inhabitants; they mostly have a complete secondary education. They have a relatively higher income in the range of 1200-2000 EUR. This segment is most willing to pay extra for Slovak products, most perceives the importance of buying Slovak products and considers the purchase of Slovak products to be a very strong support of the Slovak economy. These are therefore people with a higher income and a willingness to pay extra for Slovak products and will be fully aware of their actions in the context of the purchase of Slovak products.

The third segment consists of both genders with a slight predominance of men, of age 40-49 years and lives mainly in smaller cities with 5 to 20 thousand inhabitants; they have mostly a complete secondary education. This segment has the largest representation of university-educated respondents. This is the segment with the highest income over EUR 2,501 per month. They are not willing to pay extra for Slovak products, they do not think it is important to buy Slovak products, but they consider the purchase of Slovak products to support the Slovak economy. They are exceptional in their high income but they are reluctant to pay extra for Slovak products, they are not interested in Slovak products, but they are aware that their support helps the Slovak economy.

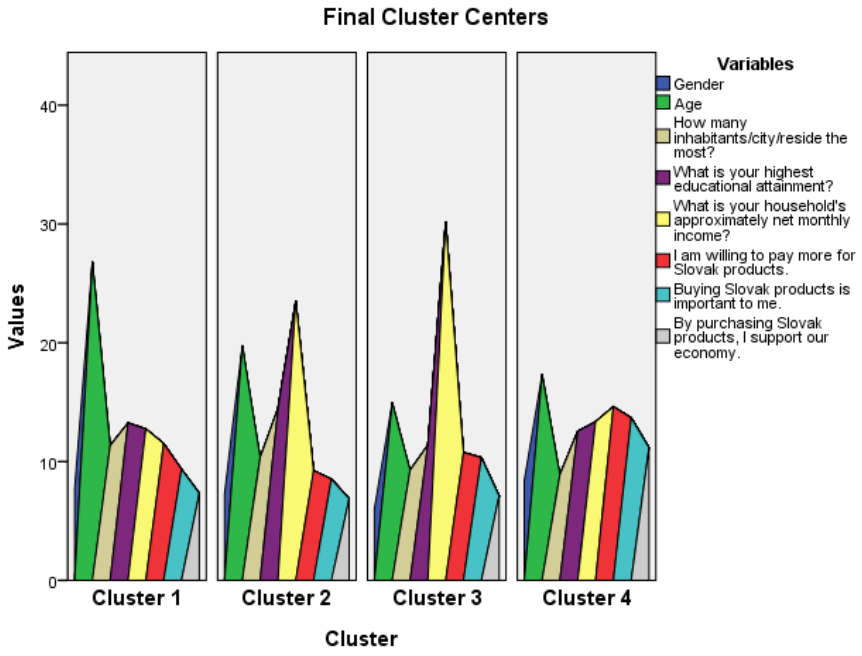
The fourth segment consists of a balanced share of both genders. It is the youngest segment of 30-39 years living mainly in villages and small towns up to 20 thousand populations. They have the lowest secondary education without a high school diploma and have the lowest income from 400 to 800 EUR per month. They are not willing to pay extra for Slovak products, it is not important for them to buy Slovak products, but they consider the purchase of Slovak products to support the Slovak economy. They are therefore young people with low incomes living in small towns and villages, with a low willingness to pay extra for Slovak products and with a low awareness of Slovak products.

Table 6: Distribution of respondents and their characteristics

CLUSTER 1	CLUSTER 2	CLUSTER 3	CLUSTER 4
310 respondents **31%** of population	**222** respondents **22%** of population	**156** respondents **15.6%** of population	**312** respondents **31.2%** of population
older people living in larger cities are aware but unwilling to pay extra from their low income	people with higher income and willingness to pay extra for Slovak products and are fully aware of their actions in the context of buying Slovak products	high income, but they are reluctant to pay extra for Slovak products, they are not interested in Slovak products, but they are aware that their support helps the Slovak economy	young people with low income living in small towns and in the village and low willingness to pay extra and with low awareness of Slovak products
Elderly conscious but unwilling to pay extra	Conscious with above - average income and willingness to pay extra	Rich following their interests	Young with low economic force and low awareness

Source: authors

The distribution of respondents into individual segments is demonstrated in Graph 1. The graph faithfully shows the distribution of respondents according to the answer coding system. The visualization reveals, for example, a stronger representation of university-educated respondents in segment 3 and an exceptionally high income in this segment. Similarly, the representation of older respondents in segment 1.



Graph 1: Visualization of cluster centroids 2020

Source: authors (IBM SPSS software)

DISCUSSION AND MANAGERIAL IMPLICATIONS

As the theoretical background showed Katt and Meixner (2020), the willingness to pay is in special product categories with value added (example of organic food). Authors applied the cluster analyses and the most positive impact on willingness to pay are linked with the consumer values, quality, labelling and locality. Our research was cowered general Slovak products with the result, that income, age and their residence could be crucial for willingness to pay more for Slovak product and play important role when it comes to awareness about the role of Slovak products and support of Slovak economy. Other factors such as gender, education had a significant importance for the cluster analysis as well and allowed us to identify 4 consumers' segments.

Interesting is the conclusion by Ubrežiová *et al.* (2019) and Kádeková *et al.* (2017) when customers tend to buy local products for the support of local economy but the price and disposable income plays important role. In context of our segment the situation seems to be similar in case of income. Segment 4 of young people with limited income is also limited in willingness to pay extra for Slovak production and do not perceive even as extremely important to notice Slovak products among the other from abroad. Segment 2 represents relatively strong purchasing power consumers with extremely high awareness and willingness to pay for Slovak products. Segment 3 looks lucrative in the purchasing power, but the topic of support of Slovak production do not consider essential at all.

The potential of support local production idea is still vivid. As the study by Memery *et al.* (2015) shows customers purchase local food more frequently with the link to the local support rather than to product quality and with this behaviour their support local worker and the local economy. This idea is sufficiently strong in each of the identified segments. Even in the low-income segment 4 of young consumers or high-income segment 3 of extremely rich consumers. Everybody They are aware of the support of the Slovak economy by buying Slovak products. A different situation is already in the evaluation of the importance of buying the Slovak products themselves. The segment 3 of the extremely rich and segment 4 of the poor does not consider this subject necessary. They are not interested in the origin of the product that it purchases. For both segments could be applied the result of the study by Taborecka-Petrovicova and Gibalova (2014) about average ethnocentric tendencies and ambivalent tendency to country of origin as a factor in consumer behaviour. We came to the same finding as *Vilčeková and Sabo* (2014) that older consumers and women prefer more than men Slovak brands as at characteristics of Segment 1. Similar results are recorded to finding by *Štarchoň and Weberová* (2016) that there is no direct link between gender and preference to Slovak brands. Here we can add that there is no relationship with willingness to pay extra for Slovak products. And woman are more sensitive to the support of Slovak economy by buying of Slovak products compared to man. Willingness to pay extra money for Slovak products is limited as the outcomes shows. Findings are similar to study by Saffu *et al.* (2010). When comparing the

results of clustering with the results presented by Horváth *et al.* (2021) we can state the compatible statement about the lower tendency towards ethnocentrism of younger generation in Slovakia. When comparing with other segments, young generation is not willing to pay more for Slovak products and the ethnocentrism (importance to buy Slovak products) is low when comparing with other segments. The only positive attitude of this generation is to the statement about support of Slovak economy by buying of Slovak products.

The practical implications for managerial purposes are connected with two main findings.

First is the dynamics of attitudes around the population in Slovakia. Comparison of consumer behaviour in seven-years period (2013 – 2020) showed that the willingness to pay more for Slovak products increased from 36% to 62.9% and almost doubled. Similarly the question of ethnocentrism about the importance of buying Slovak products showed the prevalence of positive attitudes – increased from 48.8% to 75.1%. And the last statement about the awareness of Slovak economy support when buying Slovak products increased dramatically too: from 56.8% in 2013 to 88.8% in 2020. Slovak consumer is aware and is informed how the behaviour of individual people could influence the well-being of the Slovak companies and the whole society. But this represents much more the passive behaviour. The other one – the active support represents mostly the willingness to buy and pay and of course due to the several reasons this ability counts less numerous part of population. We can state, that the change of awareness of Slovak consumers means the ability to lead them and influence towards the topics of ethnocentrism and support of local/national product consumption. The absolutely inevitable role plays activities of government, retail chains, consumer associations and individual companies. Slovak government tries to support the Slovak producers by several activities: legislation support, presence at the foreign exhibitions, but the common market of European Union is the supreme thesis, which means the principle of equal presence and chance to sell not only for Slovak but also the foreign producers at the Slovak market must be ensured. The "buy the local/national campaigns" dedicated to country of origin effect financed from public budget is therefore relatively limited. But government can influence education of young generation. And as the results showed, the youngest population is much less aware its strength towards support of national/local production – the case of identified segment 4. The topic is still important in context of fulfilment the level of self-sufficiency. Now in connection with Russian-Ukrainian crisis it looks extremely important not only for Slovakia but European Union as a whole economic block.

On the other hand, current activities of retail chains in Slovakia, represents the case how most significant retailers tries to differ in perception of customers by using of country of origin issue. Campaigns of the strongest retailers such as Lidl Slovenská Republika, v. o. s., Kaufland Slovensko, v. o. s. or Billa, s. r. o. and another retailer emphasize in their communications the strong support for Slovak products. This means the understanding of Slovak consumer behaviour

and the significance of this topic for marketing communication. Some of the retailers communicate the percentage of domestic assortment in the goods portfolio. Consumer organizations will also be activated. Due to the issues connected with import of low-quality products from abroad, and not only from Non-European regions but from the neighboring countries, the importance of country-of-origin effect gained on the importance. These associations are mostly non-government organizations and financed mostly from gifts and contributions therefore can play an important role in education of consumers. They are mainly organizations aimed at supporting of the local production of particular region.

In case of Slovak companies, it seems positive that the vast majority of the Slovak population is aware of the connection: business prosperity vs. prosperity of the whole Slovak society. But only some consumers are willing to pay a higher price for Slovak production for various reasons. We recommend focusing on the added value that the company offers to the customer. Appeal alone is not enough: "product made in Slovakia" come and support me by buying. Only 22% of the population does not need additional incentives (Segment 2). They have an income to support Slovak production and are doing so now. We identified another segment here (Segment 3), which makes up 15.6% of the population, has above-average disposable income and does not behave patriotically. They pursue their own goals and buy what is best for them, regardless of the origin of the product. And this is a challenge for Slovak companies. How to identify what is valuable for this high-income category enough to buy a Slovak product. We often encounter the claim that Slovak products are expensive compared to those produced globally. It may not be the price, but perhaps the quality and level of services provided, where flexibility and the level of consumer knowledge or relationship can be an advantage for a national/local producer. According to our research we realize that the more educated consumer, middle-aged and older, with a higher income and living in smaller cities (5-20 thousand inhabitants) is more sensitive on issue of ethnocentrism, but this is a general view of the Slovak consumer. Every business should know its customer and should identify their customer groups and assign them according to certain business criteria.

Criteria which are relevant to the type of business or industry: Segment 1 (31% of population) consists of older people living in big cities. They are educated and sufficiently aware of the need to support the Slovak products, but they are not willing to pay a higher price. Their income is a limiting factor and living in larger cities requires higher expenses. It would be appropriate for these consumers to present economic versions of the products. As they live in big cities, there should be no problem in ensuring the distribution of products in existing distribution chains. The problem may be the insufficient production required by the distribution chains. However, some distribution chains also cooperate with small producers and place their production only in selected plants in the region. They support regional products. In the case of Segment 4, we can talk mainly about the young generation (31.2% of population). They do not feel important to support local production, although they live in small towns and

villages that could prosper thanks to local producers. Therefore, they do not even have enough income to buy quality products. It is also a challenge for the government and perhaps for the retailers themselves to support the local economy of micro-regions with the potential to kick-start production of traditional products. Increasing awareness and income in this group could certainly change their consumer behaviour.

CONCLUSION

The behaviour of the Slovak consumer has been changing dynamically over the last 7 years. This dynamic could be used in the context of creating sales support for national resp. local products. It is a proven way to help the national resp. local economy and ensure a safe source of products (not just food) for the needs of the state. And, of course, achieve organization goals in case of companies both manufacturing, non-manufacturing but also retailers. Identified consumer segments show different behaviour to support Slovak products. It is possible to approach the service of each of the identified segments individually and to appeal to them sensitive topics in the context of ethnocentrism. Repeated data collection on consumer behaviour will take place in 2022. We expect quite significant change in behaviour under the influence of the pandemic and changing consumer behaviour during the Russian-Ukrainian war conflict. Rising inflation, shortages of some commodities and uncertainty will certainly affect consumer behaviour.

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IMPORTANCE OF CONTROLLING THE HYGIENIC CORRECTNESS OF HONEY AND OTHER BEE PRODUCTS IN MONTENEGRO

SUMMARY

With global environmental pollution caused by industry, traffic, agriculture, etc. the risk of contamination of honey and other bee products also increases. Environmental pollution has also increased in Montenegro, which poses a danger to the health safety of honey. That is why is very important to control the hygienic correctness of bee products. This implies monitoring the entire process of honey production, from the apiary to the end consumer. Contaminants in honey most often occur as a result of unprofessional application of agrotechnical measures, industrial production, inadequate waste disposal, application of artificial fertilizers containing cadmium and other toxic substances, pesticides containing mercury, arsenic, improperly implemented good beekeeping practices, etc. The biggest dangers for honey contamination are pesticides and means used to protect bees from varroa. In Montenegro, legal regulations set the maximum allowed concentrations of contaminants in honey (heavy metals - lead), residues of pesticides and veterinary drugs, microbiological criteria. Honey control monitoring is carried out in Montenegro every year. The results of honey monitoring in 2019 and 2020 showed that there were no non-compliant samples in terms of honey's healthiness. However, considering the mentioned factors, one should be very careful and work on increasing the number of tested samples on an annual basis.

Keywords: honey, health, safety, contamination, monitoring, Montenegro

INTRODUCTION

Montenegro has a very long tradition of beekeeping. The warm climate, relief, very rich flora enable the development of beekeeping and the production of special quality honey (Anon., 2016, Anon., 2019a). Honey is a completely natural product, a sweet substance produced by honey bees (*Apis mellifera L.*) by processing the nectar of plants, of juices from living parts of plants or by

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collecting the excreta of insects that suck juices from living parts of plants. It is a very complex mixture of more than 70 different components. The most abundant ingredients are carbohydrates, of which simple sugars - fructose and glucose and water together make up more than 99% of the composition of honey (Anon., 1981, Milošević *et al.,* 2013, Batinić and Palinić, 2014, Anon., 2014, Jovanović, 2015, Lazarević, 2016, Tanković *et al.,* 2017). Honey is used as food and medicine and its contamination can bring serious health hazards. Nutrition with honey of unknown origin and composition can have serious consequences. Insufficiently developed awareness of the dangers that occur due to improper use of chemical agents leads to large losses in beekeeping and to the fact that honey becomes a threat to human health. Harmful substances that can be found in honey originate from the environment, due to the application of agrotechnical procedures or due to poor implementation of beekeeping practices (Mahmoudi *et al.,* 2016). Increased industrialization, the development of agriculture, traffic, inappropriate waste removal, an increase in the number of illegal landfills, the burning of fossil fuels, and the development of tourism have increased the degree of environmental pollution in Montenegro as well, and thus the risk of contamination of honey and other bee products (Anon., 2017a, Bojanić Rašović, 2020b, 2020c, 2020d, 2020e, 2020f, 2020g, 2021a, 2021c). That is why is very important to control the hygienic correctness of bee products and establish traceability. This implies monitoring the entire process of honey production, from the bee farm to the final consumer.

Contaminants in honey

Food contaminants are substances that are not added to food intentionally, but are found in it as a result of environmental pollution or as a result of inadequate production, processing, packaging, transportation of food, etc. The main environmental contaminants of honey and bee products are: heavy metals (lead, cadmium, mercury), radioactive isotopes, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides (insecticides, fungicides, herbicides and bactericides), pathogenic bacteria, genetically modified organisms. The main contaminants of honey that occur as a result of inadequate beekeeping practices are: acaricides (lipophilic synthetic compounds and non-toxic compounds such as organic acids and components of essential oils), antibiotics if they are used illegally to control bee brood diseases (tetracyclines, streptomycin, sulfonamides, chloramphenicol), paradichlorobenzene, which is used to control the wax moth, chemical repellents (Bogdanov, 2006, Al-Waili *et al.,* 2012, Jovetić, 2018).

Chemical contaminants of honey

Heavy metals are significant contaminants of honey. Their importance lies in the fact that they accumulate in living organisms, are highly cytotoxic, carcinogenic, organisms do not have the ability to detoxify and thus circulate in the environment. The environment is often polluted with lead, zinc, cadmium,

chromium, copper, manganese, iron, molybdenum, arsenic, mercury. Their main source are the metal production and processing industry, traffic, remains of paint, coatings, thermal energy plants, combustion of fossil fuels, residues pesticides, mineral fertilizers, etc. Lead enters the nectar or honeydew directly from the air. Cadmium in nectar and honeydew is transported from the soil to the plant, and through the plant to the bee. An increased concentration of heavy metals, especially mercury and chromium, was also found in the pollen (Jovetić, 2015, Tanković *et al.*, 2017). *Polycyclic aromatic hydrocarbons* (PAHs) are liposoluble organic compounds that have two or more condensed aromatic rings in their structure. They are created during pyrolytic processes, especially incomplete combustion of organic matter, processing of coal and crude oil, combustion of natural gas, heating, incineration of waste, traffic, smoking. These compounds exert mutagenic and carcinogenic effect. The most toxic is benzopyrene, which is most often used as an indicator of the presence of these compounds in the environment. PAHs reach honey through air, water, soil, as well as during the process of fumigation of bees in the hive and honey processing (Sofilić, 2014). Elevated levels of PAHs was determined in samples of honey and other bee products from urban and industrial areas. Residues of polycyclic aromatic compounds were found in honey produced near oil factories. The content of PAHs in pollen samples is significantly higher than in honey samples, because the average proportion of lipids in pollen is even and up to 22.4%. A special group of pollutants is the organochlorine group compounds, the so-called *persistent - long-term pollutants* (Persistent Organic Pollutions, POPs). These compounds are poorly soluble in water, and they dissolve very well in fats, which results in their bioaccumulation in the fatty tissues of living organisms. They are toxic to living organisms. They are transported over long distances by water and air, which is why they are widely distributed even where they have never been used. They are carcinogenic. The most widespread from this group are: polychlorinated biphenyls (PCB), dioxins (polychlorinated dibenzo-p-dioxins–PCDD), furans (polychlorinated dibenzofurans–PCDF) and organochlorine pesticides (OCP). Polychlorinated biphenyls (PCBs) are organic chemicals that originate from transformer and motor oils, coolants etc. They were produced until 1980, but they are still present in the environment and endanger bees and their products. Their content in wax is higher than in honey. One of the most dangerous environmental pollutants that represent a particular problem are dioxins. They are created as a result of inadequate incineration of municipal and medical waste and as by-products of the chemical industry. The air serves as a medium for the transfer of dioxin molecules and particles, which then fall to the ground, and in this way the bees bring them into the hives via pollen. If dioxins are found in honey, honey is not allowed for consumption (Jovetić, 2015).

The control of honey for the presence of *radioactive substances* is also very important. The main radioactive isotopes found in honey are 40 K and 137 Cs - the first is of natural origin, and the second is from the Chernobyl nuclear power plant in 1986. According to literature data, in the early sixties of the last

century and during the accident in Chernobyl in 1986, the area of the Republic of Croatia was contaminated by the processes of dry and wet deposition from the atmosphere, as well as by the radioactive isotope cesium 137 Cs. Deposited cesium is absorbed on soil particles over time, and partly penetrates into deeper layers and reaches the zone of the root system of plants. Like other biogenic elements, cesium is incorporated into plants through the root system. More than fifteen years after the Chernobyl accident, 137 Cs was still found in some types of honey, such as coniferous honeydew and chestnut honey (Barišić *et al.*, 2005).

Biological contaminants of honey

The presence of microorganisms in honey can affect its quality and health safety. Microorganisms found in honey and honeycomb are bacteria, molds and yeasts, and they come from bees, nectar or from external sources. Pollen, intestinal tract of bees, man, equipment, containers, wind, dust are possible sources of contamination by microorganisms. Moisture and temperature are of great importance for the growth of microorganisms in honey. Microorganisms that can be found in the intestines of bees are yeasts (1%), gram-positive bacteria (*Bacillus, Bacteridium, Streptococcus* and *Clostridium spp*.) 27% and gram-negative bacteria (*Achromobacter, Citrobacter, Enterobacter, Erwinia, Escherichia coli, Flavobacterium, Klebsiella, Proteus* and *Pseudomonas*) 70%. Most bacteria and other microorganisms can not grow or reproduce in honey. Honey has an antimicrobial effect and prevents the growth of many microorganisms. In addition, honey has a low water activity, which leads to the prevention of the reproduction and survival of bacteria. However, several types of pathogenic bacteria have been found in honey. The finding of a large number of vegetative forms of bacteria that can not reproduce in honey indicates fresh contamination. Different types of vegetative forms of bacteria in honey stored at 20°C decay within 8-24 days. However, spores of microorganisms can survive in honey at a low temperature. *Bacillus cereus, Clostridium perfringens* and *Clostridium botulinum* spores in honey stored at 25°C remain viable. Honey, as a substance with a high concentration of carbohydrates and a low pH value, adversely affects the development of bacterial spores. Bacterial spores and molds are brought to the hive by bees along with pollen and can be found in all bee products (Al-Waili et al. 2012). To destroy the spores, a temperature of 130 °C, increased pressure and a time period of three minutes are necessary. Honey is not subjected to these conditions (the exception is industrial honey), because this would damage the biological and physical-chemical properties of honey. There is a risk of botulism in infants if they consume contaminated honey. Honey that has not been tested and sterilized should not be used in the diet of infants or applied to wounds. Yeasts (genera *Candida, Saccharomyces*) and molds (genera *Penicillium, Aspergillus, Mucor*) can also be found in honey. *Mycotoxins* can be found in honey as a product of mold metabolism. Molds of the genus *Aspergillus* (*A. flavus* and *A. parasiticus*) produce aflatoxins, of which the most toxic is aflatoxin B1. Aflatoxins have a mutagenic and carcinogenic effect - they cause

liver cancer in humans. The source of mold contamination of honey is the intestinal content of bees, hives and pollen. Molds from the genus *Aspergillus* were also found in the gut of bee larvae. Pollen is considered to be the main source of microbial contamination of honey. Yeasts, toxicogenic molds, etc. were also found in it (Mahmoudi *et al.*, 2016). Honey produced from the flowers of some plants can cause intoxication and various symptoms such as dizziness, weakness, sweating, nausea, vomiting, hypotension, shock, and arrhythmia and death can occur. Some substances that are toxic to humans are not always toxic to bees. The nectar of certain plants contains poisonous substances. Consuming honey contaminated with the nectar of the *toxic rhododendron plant* leads to poisoning that can be life-threatening. In a mild form of poisoning, nausea, vomiting, excessive salivation and dizziness occur, and in a severe form, heart failure occurs. In addition to this plant, the toxic effect of honey obtained by collecting nectar from the flowers of plants such as *Andromeda polifolia* (wild rosemary), *Kalmia latifolia* (kalmia), plants of the genus *Melicope* in New Zealand, *Nerium oleander* (oleander) in the Mediterranean region, etc. is known (Dugasa *et al.*, 2019). In the United States and Canada, *genetically modified plants* are commonly grown and accepted by the public, while in the European Union there is strong opposition against the consumption of food containing genetically modified organisms (GMOs). In the European Union, labeling of food containing genetically modified organisms in an amount greater than 1% is mandatory. The PCR method makes it possible to detect the presence of only a few grains of pollen originating from a genetically modified plant. Bee pollen can be significantly contaminated, while honey, which contains less than 0.1% pollen, would not exceed this total GMO allowance of 1%. In countries where genetically modified oilseed rape and corn are grown, it can be a problem for beekeeping.

Residues in honey

Residues are the remains of intentionally used substances (pesticides and veterinary drugs) in the food production chain with the aim of improving production. *Pesticide residues* that can be found in honey can be acaricides, organic acids, insecticides, fungicides, herbicides and bactericides. Residues of pesticides and veterinary drugs lead to health problems such as malaise, eye pain, skin and respiratory system problems, gene mutations, cell damage, cancer, fetal malformation, chromosomal abnormalities and weakened immunity in humans. As a result of pesticide poisoning in humans, the cessation of the function of the vital centers, paralysis of the respiratory center, heart failure, pulmonary edema occur (Nzeh *et al.*, 2020., Jovanov *et al.*, 2015). Uncontrolled application of pesticides causes contamination of the environment, animals and people. Over 150 different pesticides were found in the hives. The most dangerous are organochlorine pesticides, which belong to persistent organic contaminants (POPs), because they break down slowly, accumulate, pollute soil, water, air, crops, and the living world. They have the ability to accumulate in the organisms

of plants and animals, so their long-term and uncontrolled use leads to a very harmful effect on the living world. Hexachlorocyclohexane (HCH) and its isomers are the most frequently found pesticides in honey, followed by dichloro-diphenyl-trichloroethylene (DDT) and its isomers (Dugasa *et al.*, 2019). Our country is a signatory to the Stockholm Convention on POPs (Anon., 2013b), which the use of organochlorine pesticides is prohibited due to their deposition in fatty tissue and a long half-life. The use of neonicotinoid pesticides is a risk for honeybees. There is a special risk from three types of neonicotinoids: clothianidin, imidacloprid and thiamethoxam. If the use of pesticides is carried out without control and in an inadequate way, to severe consequences - endangerment of bee colonies and contamination of their products. The most important pollutants of honey are substances that used to control bee diseases, primarily the causative agent of varroosis and american plague of bee brood. Synthetic acaricides are mostly fat soluble and stable in beeswax. After treatment, acaricides accumulate in wax and to a lesser extent in honey. Contamination of honey and other products with acaricides used to protect bees is, therefore, even more significant than contamination from the environment. They lead to direct contamination of the product. Honey is more often contaminated with pesticides than pollen, but the highest concentrations were found in pollen (Jovetić, 2018). The acaricide amitraz is rapidly degraded in the hive to metabolites, some of which are more toxic than amitraz to both humans and bees. In addition to honey, amitraz also contaminates wax, because it enters the structure of the wax as a liposoluble substance. Such contaminated wax leads to negative consequences after its use in medicine and cosmetics. Amitraz residues in honey are not allowed in some countries such as Italy, France, Japan and Germany (Ivanović *et al.*, 2021). Amitraz is according to the Regulation of the European Commission no. 775/2004 prohibited for use and circulation in the European Union. (Nzeh *et al.*, 2020, Bojanić Rašović, 2021b). The monitoring of pesticide residues in honey, wax and bees helps to assess the potential risks of these products to human health and provides data on the extent of pesticide treatment of agricultural crops in the vicinity of beehives. The main contamination risks for different bee products are: antibiotics for honey and royal jelly, lipophilic acaricides for beeswax and propolis, pesticides for pollen. *Residues of antibiotics* can occur due to treatment against american bee brood plague and european bee brood plague. Antibiotic residues have a relatively long half-life and can have direct toxic effects on consumers. They are also carcinogenic, cause reproductive disorders, and have a teratogenic effect. Long-term exposure to antibiotic residues leads to the appearance of resistance of pathogenic bacteria to antibiotics. The European Union as well as national regulations prohibit the use of antibiotics in the treatment of bee diseases. In most EU countries, no maximum allowed amounts of antibiotics are prescribed, which means that honey containing antibiotics is not allowed to be sold. However, some countries, such as Switzerland, Great Britain and Belgium have established maximum permitted amounts of between 0.01 to 0.05 mg/kg for each group of antibiotics (Vapa-Tankosić and Lekić, 2017).

Some beekeepers use *para-dichlorobenzene* (PDCB) to control the wax moth. This substance contaminates commercial beeswax and honey. An even more toxic substance is naphthalene, which is also used to control the wax moth and its residues have been found in honey. Means and paints for the protection of wood and beehives should not contain insecticides and fungicides, as this could lead to contamination of honey. Chemical repellents can also be a source of honey contamination. Remnants of phenol, a widely used repellent, were discovered in honey. Since it can also be found naturally in honey, the measured concentrations should be carefully analyzed.

Awareness of the severity of these dangers should motivate beekeepers and agricultural producers to create a joint pesticide management plan, in order to maximally avoid their negative impact on bees, but also on all living things and the environment. Introducing organic beekeeping is the best way to avoid the mentioned sources of contamination. To that end, beekeepers should be motivated and supported for practicing organic beekeeping. Organic beekeeping respects the laws of nature, does not disturb the natural balance and preserves the environment. It represents a return to tradition and healthy technologies, uses natural materials, which increases the bee's resistance. The priority of organic beekeeping is to achieve high quality and product safety. Organically produced honey, which is free of harmful chemical residues, has a beneficial effect on human health with its nutritional value and taste.

The importance of implementing good production and good hygiene practices in obtaining healthy honey in Montenegro

The lack of hygiene in production leads to a violation of the health safety of honey. Risks of dust contamination occur if containers with honey are kept open, if attachments are stored in unhygienic rooms, if a rusty and unclean tool is used to remove the lids, etc. Contamination of honey can also occur during processing, through equipment and tools. Storing honey in inappropriate containers can also lead to the appearance of undesirable heavy metal residues. Materials from which beekeeping equipment is made (aluminum, stainless steel, galvanized steel) can release contaminants such as aluminum, cadmium, cobalt, chromium, copper, iron, lead, nickel, zinc into the honey. Production equipment, as well as honey storage containers, must be made of materials specially intended for food. During the storage of honey, inorganic and organic components can diffuse from the inner surfaces of paraffinized, corrosive and painted containers and contaminate the honey (Bojanić Rašović 2020a, Bojanić Rašović 2020b, Bojanic Rasovic 2022). The carcinogenic compound semicarbazide was found in an amount of 0.003 up to 0.005 mg/kg honey and comes from lids for closing glass jars. Lids for jars are made of metal sheets, on which a sealing compound is applied or various washers are placed that ensure the tightness of the closed jar. One of the most commonly used lids is the so-called twist off (screw cap). EC Directive 2007/19/EC prohibits the use of azodicarbonamide in the manufacture of plastic materials and articles that come into contact with food. This compound

decomposes at high temperatures to semicarbazide. This information points to the importance of using proven, certified packaging intended for packing and storing food (Bogdanov, 2006). Should apply the HACCP system in order to ensure healthy honey and other bee products. It is based on the principle of hazard analysis and determination of critical control points for controlling and preventing the occurrence of these hazards. The basis for establishing this system is to consider all potential dangers and health risks from these dangers. The HACCP system - a system of hazard analysis and critical control points is useful for beekeepers, entities in the food business, veterinarians, and veterinary inspectors in the planning and implementation of controls. The bee pest control strategy should be based on eliminating the use of synthetic chemicals, so that the bees are healthy and the products are healthy. Non-toxic natural substances such as thymol and organic acids should be used. Given that it is soluble in fats, the remains of thymol occur in larger quantities in beeswax. Residues of thymol evaporate during wax storage. If thymol treatments are carried out throughout the season, its residues in honey can be in such a quantity that they can lead to a change in the taste of honey, which is not allowed according to international regulations on honey. That is why you should not exceed the limit of thymol content in honey of 1.1–1.5 mg/kg. Oxalic and formic acids are natural constituents of honey and are widely used in varroa control. They have the so-called GRAS status (generally recognized as safe) and maximum permitted amounts in honey are not prescribed for them. Single or repeated treatments with oxalic acid do not lead to the accumulation of residues in honey. It was shown that after treatment with formic acid, the concentrations of the residues of this acid in honey were within the limits of natural concentrations. Proper long-term use of formic acid does not lead to an increase in its concentration in honey, but if it is used unprofessionally, the content of its residues can be high and change the taste of honey (Bogdanov, 2006). The control of honey and bee products is very important in order to protect the health of consumers, but also to ensure healthy competition of producers on the market. Given that a prerequisite for honey production is an ecologically clean environment, the big challenge in the future for beekeepers will be how to get health-safe honey of good quality. For honey as a food with a special reputation, according to EU regulations, a zero level of pesticide residues is prescribed, which is a big problem for beekeepers in countries where control is rigorous, given that pesticides are widely used in the treatment of fruits, vegetables and other agricultural crops (Bogdanov, 2006).

Legal regulation in the field of health control of honey in Montenegro

Monitoring of residues and contaminants is of great importance for the safety of honey in Montenegro. It is carried out in accordance with the Rulebook on residue monitoring according to the annual Program of Food Safety Measures (Anon., 2017b, Anon, 2021). Samples are taken in any part of the honey production chain, in such a way that the origin of the honey can be determined. The number of samples that should be taken every year is at least 10 samples per

300 tons of annual production for the first 3000 tons of production, and one sample for every additional 300 tons. The distribution of samples is done by testing 50% of the samples for the presence of antibacterial substances, including sulfonamides and quinolones (group B1) and carbamates and pyrethroids (group B2), while 40% of samples are tested for organochlorine compounds and polychlorinated biphenyls, organophosphorus compounds and chemical elements (group B3), while 10% of samples are distributed based on experience, with special attention addresses mycotoxins (Anon., 2017a). During monitoring, two samples of honey were examined in 2019, and three samples of honey were examined in 2020, with all nor were the samples harmonized according to legal regulations (Anon., 2019a, Anon., 2020). In relation to the information that honey production in Montenegro in 2017 was 390,000 kg, this means that the number of honey samples taken for monitoring should be higher (Anon., 2019d). Our Regulation on the Maximum Permitted Amounts of Contaminants in Food (Anon., 2019c) prescribes a maximum permitted amount of lead of 0.10 mg/kg of honey. In addition to lead, regulations in Serbia also define maximum permissible amounts for cadmium, arsenic, zinc, iron and copper (Anon., 2018b). Our regulations do not prescribed maximum permitted amounts of PAHs for honey and other bee products, which means that the tolerance for PAHs in these products is zero (Anon., 2019a, Ivanović *et al.*, 2021). The maximum permitted amounts of polycyclic aromatic hydrocarbons (PAHs) are in the European and domestic regulations defined only for foods containing fats and oils and where contamination may occur during the smoking or drying process. Maximum permitted amounts in food were determined for four PAHs: benzo[a] pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene. Our Rulebook does not specifically prescribe the maximum permitted amount of pesticides in honey, which means that honey must not contain more than 0.01 mg of pesticide/kg of honey (Anon., 2015). The Rulebook on Maximum Permitted Amounts of Residues of Pharmacologically Active Substances of Veterinary Medicines in Products of Animal Origin (Anon., 2018a) prescribes the maximum permitted amount of amitraz and its metabolites containing 2,4-DMA in honey, which is 200 μg/kg of honey. The maximum permitted amount of coumaphos in honey according to the same regulation is 100 μg/kg of honey. Antibiotic residue concentrations are not prescribed by the Rulebook (Anon., 2018a), which means the regulations of the European Union, as well as our regulations, do not determine the maximum allowed amount of antibiotics, which means that the level of antibiotics in honey must be zero and that honey containing antibiotics is not allowed for sale. The regulation on maximum permitted amounts of contaminants in food (Anon., 2016) does not prescribe maximum permitted amounts of mycotoxins in honey, which means that the tolerance dose for mycotoxins in honey is zero. According to our Law on Genetically Modified Organisms (Anon., 2008) and the Regulation on the conditions and manner of using genetically modified food or animal feed (Anon., 2019b) food containing more than 0.9% genetically modified organisms must be marked on the

declaration. According to the aforementioned regulation, the cultivation of GMOs in the open is prohibited, and for the cultivation of GMOs in closed conditions, special permits are required from the State and the National Council for GMOs. According to the Guide for Microbiological Criteria for Food Safety of Montenegro (Anon., 2013a), the recommended microorganisms for testing honey and other bee products, as well as honey-based products, are aerobic mesophilic bacteria, enterobacteria, sulfite-reducing clostridia, yeasts and molds. These microorganisms show the degree of hygiene in the process of production, packaging and storage of honey, they can lead to spoilage of honey, and some are pathogenic and can lead to diseases. These parameters refer only to production hygiene criteria. Of the five tested samples, it is acceptable that two samples can have a total number of aerobic mesophilic bacteria between 10^3-10^4 cfu/g, one sample can have a total number of enterobacteria of 10-10^2 cfu/g, and all five tested samples must have less than 10 cfu of clostridia in one gram of tested honey sample. Of the five samples tested, it is acceptable that one sample can have 10-10^2 cfu of yeasts and molds in one gram of honey sample. When it comes to other bee products and honey-based products, out of five tested samples, it is acceptable that two samples can have 10^4-10^5 cfu of aerobic mesophilic bacteria/g of honey, one sample 10^2-10^3 cfu of enterobacteria/g, one sample 10-10^2 cfu clostridia/g, one sample 10^2-10^3 cfu yeasts/g and one sample 10^3-10^4 cfu molds/g honey sample. These criteria, therefore, do not refer to finished products intended for the market, but to hygiene criteria during production. If these criteria are not met, the hygienic measures of production of honey and bee products must be improved (Anon., 2012, Anon., 2013a).

CONCLUSIONS

In Montenegro, there are good climatic conditions for the development of beekeeping and the production of high quality honey. However, due to increased environmental pollution from industrial production, traffic, agriculture, improper waste disposal, improper treatment of bees etc., there is a danger of contamination of honey and other bee products. This is why the role of primary production is crucial in food safety management and hazard analysis. It is very important to work on implementing good beekeeping practices and on the continuous control of honey production throughout the entire production chain. Annual monitorings are carried out regularly, which examine the honey for the presence of contaminants and residues of pesticides and antibiotics. The monitoring results in 2016 and 2017 showed that the tested samples were in compliance with the legal regulations in Montenegro. The number of samples covered by monitoring should be increased in accordance with legal regulations.

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GROUNDWATER QUALITY FORECASTING USING MACHINE LEARNING ALGORITHMS: CASE STUDY BERRECHID AQUIFER, CENTRAL MOROCCO

ABSTRACT

In order to provide a recommendation on the quality of groundwater in the region of Berrechid, Morocco, we analysed the concentration of conductivity as one of the main measures to identify the salinity of the water. We applied artificial intelligence models for predicting the conductivity of water while analysing several physical parameters as input parameters of the models. To achieve this purpose, we exploited and evaluated the Random Forest (RF), Support Vector Regression (SVR), and k-nearest neighbour models using 400 data samples related to ten groundwater quality parameters in the Berrechid aquifer, Morocco. The results revealed that the overall prediction performance of the RF models is higher than the SVR and KNN models. Overall, the developed models are able to predict conductivity with high accuracy. The approaches developed in this study are promising for real-time and low-cost prediction of groundwater quality by using physical parameters as input variables.

Keywords: Groundwater quality, Artificial Intelligence, Random Forest, Support Vector Regression, k-Nearest Neighbour's.

INTRODUCTION

Groundwater is one of the most important water sources for agriculture, but also for other industries. Groundwater supplies nearly two-thirds of the world's population with drinking water and other domestic uses (Adimalla *et al*., 2018). More than 1.5 billion people worldwide rely on groundwater for basic needs such as drinking and irrigation (Adimalla and Li 2019). In addition, 946 million people lack access to adequate drinking water and have sanitation practices that are not clean (UNICEF and WHO 2015). Water availability is one of the most

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challenging tasks of the 21st century (Adimalla and Qian 2019). Water demand has been increasing due to population growth, intensive agriculture, urbanization, and industrial expansion in recent years. However, climate change, anthropogenic and natural activities have led to a decrease in groundwater quantity and degradation of its quality, making it unsuitable for domestic consumption, irrigation, and industry (Du *et al*. 2016; He *et al*. 2016; Li *et al*. 2018; Khanoranga and Khalid 2019; Su *et al*. 2019). Recent studies have estimated that 3.5-4.4 billion people are expected to live with the problem of water supply in 2050 due to climate change and increasing human water demand (Hanasaki *et al*. 2013; Wada *et al*. 2014). Therefore, assessment and prediction of groundwater quality is necessary for effective management and to keep pollution levels within allowable limits (Meguid 2019; Najah Ahmed *et al*. 2019). Conventional process-based modelling methods provide relatively accurate predictions for water quality parameters. Because of this, groundwater experts have focused their efforts on determining groundwater quality and suitability for drinking, household, irrigation, and industrial uses (Adimalla and Venkatayogi, 2018; Roy *et al*. 2018; Ehya and Saeedi, 2019; Mukate *et al*., 2019; Das *et al*., 2020).

Water quality is based on many indices and parameters adopted to meet water needs. Several studies have focused on index and statistical-based approaches. Recently, Adimalla *et al*. (2018) used Water Quality Index (WQI) to determine the adequacy of water quality for irrigation. Rao et al. (2018) used a groundwater pollution index (GPI) to assess groundwater quality for drinking purposes. Dutta et al. (2018) effectively demonstrates the use of WQI and multivariate statistical techniques to obtain simpler and more meaningful information about water quality and identify pollution sources. These methods have been applied to support groundwater quality research and promote various groundwater quality assessment and management strategies, all with the innovative and intelligent methods of reasonable cost to assess the state of groundwater quality. To this end, prediction-based approaches can be useful tools to overcome challenges in groundwater planning and management. Nowadays, application of artificial intelligence (AI) techniques has increased in many fields. In the environmental sciences, researchers proposed intelligent and adaptive dynamic water resources planning to preserve the water environment (all surface water, groundwater and wetlands) in urban areas (Xiang *et al*. 2021). Other studies conducted a systematic review of the literature on applying different types of artificial intelligence models to enable better monitoring of surface water quality (Ighalo *et al*. 2021). In the area of groundwater quality assessment, researchers investigated the performance of a few artificial intelligence techniques, including particle swarm optimization (PSO), a naive Bayes classifier (NBC) as a simple "probabilistic classifiers", and a support vector machine (SVM) with the purpose of predicting the water quality index (Agrawal *et al*. 2021). Other research scrutinize artificial intelligence technologies for assessing source water quality, disinfection, and membrane filtration, including monitoring

and identifying source water contaminants (Li *et al*. 2021). Researchers highlight a new approach based on integrating deep learning and feature extraction techniques to improve water quality classification (Dilmi and Ladjal, 2021). However, several factors are considered in a prediction model, including the nature and number of predictors used. Thus, a proper selection of input variables is required to increase the efficiency of Machine Learning (ML) models.

Morocco is a semi-arid Mediterranean country where surface and groundwater resources are essential for socio-economic sustainability. Consequently, aquifers are heavily exploited to meet increasing agricultural, industrial, and demands of local population. In addition, accelerated industrial operations, rapid population expansion, and agricultural intensification have led to significant groundwater depletion and degradation (Malki *et al*., 2017). Groundwater depletion is a significant problem in Morocco and is of particular concern to water managers. Rapid declines in groundwater levels (0.5 to 2 m per year on average) is generally caused by low groundwater recharge, marine intrusion, and excessive expansion of agricultural activities (Fadili *et al*., 2015; Najib *et al*., 2016; Ait Kadi and Ziyad, 2018; Alabjah *et al*., 2018; Mountadar *et al*., 2018; Bilali *et al*., 2021; Moukhliss *et al*., 2021; Zeynolabedin *et al*., 2021). Studied areas represent typical cases and are strongly impacted by climate variability, anthropogenic activity, and marine intrusion. In this case, the most important problems affecting groundwater recharge are intermittent river flow, decreasing reservoir capacity, the release of water by dams, removal of groundwater by pumping, soil salinity in irrigated areas. Despite these problems, the area remains an excellent example of effective quantitative and qualitative management of groundwater resources in integrated water resources management.

The main objectives of this study are: (1) predicting water conductivity using several physical parameters as model input parameters; with (2) comparing the performance of four models, including Random Forest, Support Vector Regression (SVR) and Machine learning (ML).

MATERIAL AND METHODS

Study area

The Berrechid aquifer is located in the Atlantic coastal basin between Rabat and Azemmour. It is around 10,470 km^2 in size (Royaume du Maroc, 2003). This aquifer is situated in the south of Casablanca. It differs from other aquifers in the region due to its enormous surface area of around 1,500 km^2. It is part of the quadrilateral formed by Settat, El Gara, Mediouana, and the center of Bouskoura (Figure 1).

This is a semi-arid region with annual rainfall ranging between 280 and 320 mm, with more than 90% falling between October and April. The temperature varies from 6.5 °C in January to 38 °C in August (Lyazidi *et al*., 2003; El Gasmi *et al*., 2014).

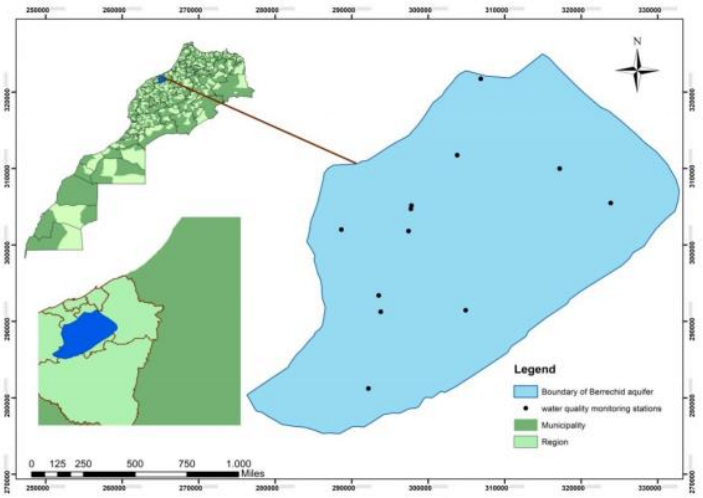


Figure 1. Study area and monitoring station

The basin is endorheic, having no outflow to an external body of water such as a river or ocean, and only losing water through evaporation or seepage into the ground; it is naturally fed by rainfall and streams that enter through the south and disappear below the plain (Moullard, 1960). The basin is flat in topography, with altitudes and slopes varying from 140 m and 0.2 % in the North to 350 m and 0.8% in the South (Naïma *et al*., 2015). In semi-arid climatic contexts, numerous geological formations were formed, combined with ancient subsidences and sedimentations spanning from primary to quaternary deposits. Nevertheless, the lithostratigraphic succession of these formations is as follows:

1) Primary, the bedrock consists of shale interbedded with layers of quartzite and sandstone, with 150 m thick outcrops Siluro-Devonian and Acadian Green to the SE and NW (El Mansouri, *et al*, 1992);

2) Triassic: Saline and siliciclastic red clays are interbedded with evaporite and basalt in these deposits. They are distributed in the eastern part of the aquifer (Bensalah *et al*., 2011);

3) The Infra-Cenomanian: with a total depth of about 40 m, including detrital red clays and gypsum abundant in the deposits. Layers of limestone and white succeeded some layers of the yellow marl conglomerate;

4) The Cenomanian, predominantly made up of dolomitic limestone and yellow marl, with green marl intercalated across a thickness of roughly 120 m (Ruhard 1975);

5) Pliocene that set the Berrechid aquifer system made up of sandstone, sand, sandy limestone, and small conglomerates with a total thickness of 5 to 40 m (Droubi *et al*. 2008);

6) Quaternary: The dominating facies is characterized by silts and conglomerates, which are followed by red silty clays, pebbles, and gravels with thicknesses varying from 0 to 50 m. As far as water quality is concerned, this

water table is classified into three hydro-geochemical facies (Na-Cl; Na-Mg-Ca-Cl; Ca-Mg-HCO_3-Cl). This region is marked by fertile soils and productive water tables, which causes the depletion of the water tables and the degradation of water quality, the latter becoming non-compliant with the consumption standard (El Ghali *et al*., 2020). Farmers mainly consume water for market gardening and livestock (Ouassissou *et al*., 2019). Water quality is monitored by the Bouregreg and Chaouia Basin Agency through fourteen monitoring stations.

Machine learning models

The models used are the Random Forest, Support Vector Regression (SVR), and KNN approaches.

Random Forest. It is a supervised machine learning algorithm that is hugely used in many classification and regression problems. It consists in building decision trees on different samples and takes their majority vote for classification and the mean in the case of regression. The concept of the sample with training data replacement is considered in each decision tree, also based on the bagging (Random forests: from early developments to recent advancements). The Random Forest offers several advantages: (a) Generally, the accuracy of Random Forest is considerably better than other types of decision trees. (b) The speed of Random Forest is high compared to bagging and boosting. (c) The estimates are significant in terms of error and correlation.

A large number of decision trees are the subject of the Random Forest. In both the construction and selection processes of the sample subsets, the random process is integrated to guarantee certain independence of each decision tree, thus improving the accuracy and performance of the model (A Review on Random Forest: An Ensemble Classifier). The following diagram (Figure 2) gives a brief description of the Random Forest model (K. Liu *et al*., 2021):

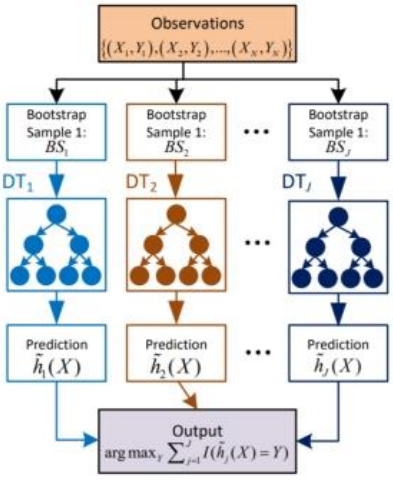


Figure 2. Random Forest process

K-nearest neighbors – KNN. The algorithm is a Machine Learning algorithm (Peterson 2009) belonging to the class of simple supervised learning algorithms, easy to implement, and can solve classification and regression problems. The KNN algorithm consists of two primary steps:
Step 1: Select the number K of neighbors.
Step 2: Calculate the distance, the Manhattan distance or the Euclidean distance.
Step 3: Take the K nearest neighbors according to the calculated distance.
Step 4: Among K neighbors, count number of points belonging to each category.
Step 5: Assign the new point to the most present category among K neighbors.

Several methods can be effective in determining the value of K. Generally, several values of K are tested to obtain the most effective value. Usually, the K number is increased. This defines the nearest neighbor region and brings more clarity and transparency to the results. However, when the data dispersion is a problem, the value is difficult to specify. The diagram of the KNN algorithm summarizes all the steps (Figure 3).

Figure 3. KNN process

Support vector regression SVR. It is an evolution of support vector classification to perform regression tasks. To perform this task, the ε-insensitive loss function is implemented to analyze the data.

Data collection and analysis. As part of this research, four hundred quality samples were collected at the following monitoring sites in the Berrechid aquifer. The sample methodology was carried out twice in the spring and summer during irrigation pumping. The wells varied in depth from 40 to 140 meters. All of the wells are utilized on a regular basis for residential usage, irrigation, and industrial purposes.

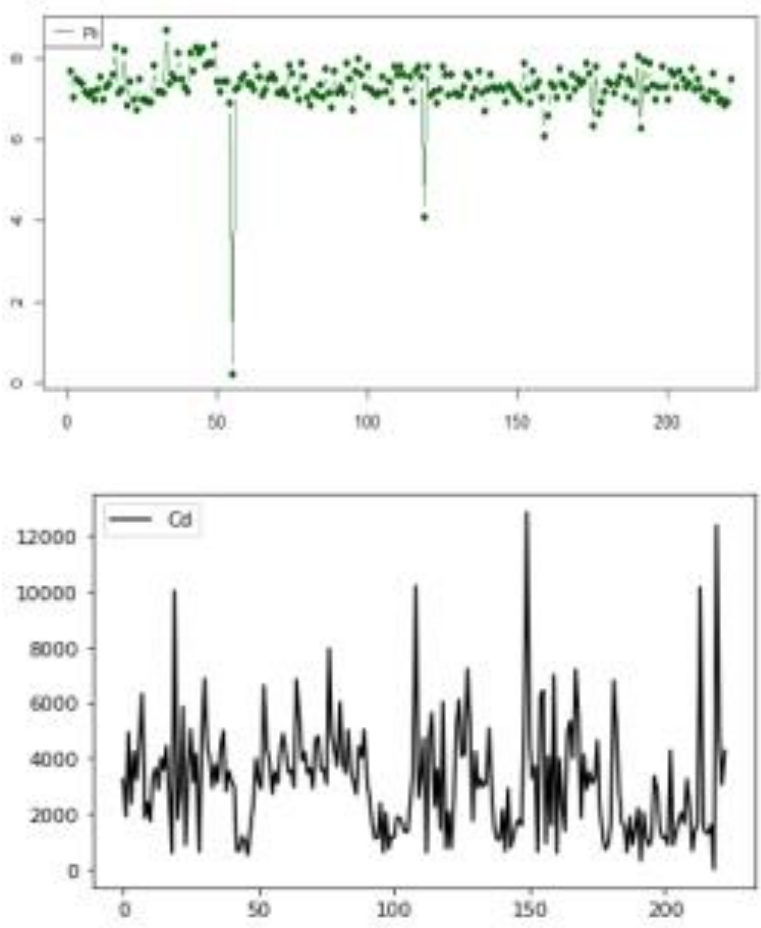
For sample collection, we utilized one-liter polyethylene bottles that had been prewashed and labeled. The following parameters were measured in these samples: Electrical Conductivity (EC), Chlorides (Cl^-), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), pH, Sulfate (SO_4^{2-}), Sodium (Na^+), Potassium (K^+), Carbonates (CO_3^{2-}), and Bicarbonates (HCO_3^-). The collecting points' location coordinates (X, Y coordinates) were determined using a portable global positioning system (GPS). The samples are sent immediately to the laboratory for analysis.

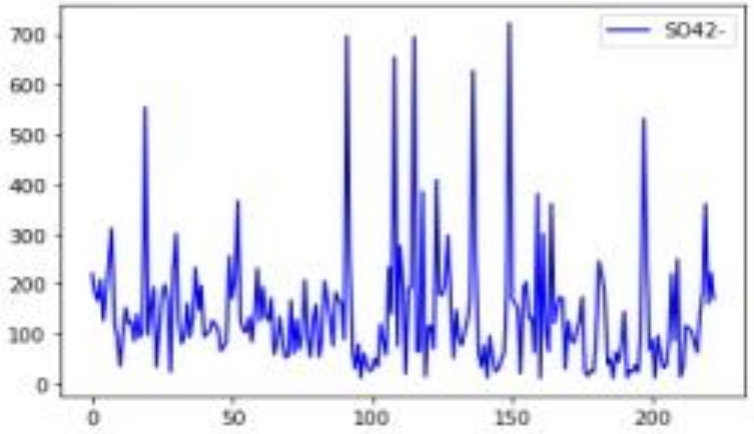
Conductivity was measured with an intelligent conductivity meter YK-2001PH. The determination of chloride was achieved by the MOHR method, according to AFNOR 90-014. The determination of bicarbonate and carbonate was carried out by the titration method in the presence of H_2SO_4 (0.02 N), a solution of NaOH (0.1 N), phenolphthalein and methyl orange indicator. The sulfate determination was performed using the spectrophotometric method (Shimadzu UV 1800 model). The determination of potassium was conducted by a flame photometer (Elico flame photometer model CL 22 B) and KCl reagents. Sodium was determined by a flame photometer (Elico flame photometer model CL 22 B) and by NaCl reagent. Calcium was determined by EDTA titrimetric analysis. The magnesium determination was performed by TH and Ca concentration. The solutions' hydrogen potential (pH) was determined by a "Accumet Basic AB15" pH meter.

Data processing and model performance evaluation methods. From an analysis of all the parameters, there are aberrant values on almost all the parameters. Therefore, it is important to analyze the data before entering it. Indeed, data exploration and cleaning are among the main steps to obtaining efficient models. Moreover, in ML model development, data mining and cleaning are the significant steps to obtaining accurate and reliable models. In this study, data pre-processing was performed in several steps:

Step 1: Checking for outliers and missing values. In our database, there are two scenarios. The first scenario concerns outliers. An outlier is a value or observation that is "distant" from other observations made on the same phenomenon, i.e. it contrasts significantly with the "normally" measured values. Therefore, any value that appears to be an outlier in the database should be removed. They can either be replaced for missing values the row related to the missing data can be deleted. Since there are only two missing values in our case, we chose to delete the other data related to these missing values.

Step 2: A complete description of the different parameters involved. This step consists of making a complete description of all the parameters we consider in creating the machine learning models. This first description includes the evolution of each parameter and the min, max, average and median values, etc. A general view, but also diagrams showing the range for each parameter. This is achieved firstly through graphs visualizing the data for each variable. Figure 4 illustrates the following variables: CE, pH, Cl^-, SO_4^{2-}, Na^+, K^+, CO_3^{2-}, HCO_3^-, Ca^{2+}, Mg^{2+}. In the conductivity, for example (CE), there are places where the conductivity is significantly high and where the conductivity remains very limited. This creates ups and downs throughout the graph. This applies to all variables. However, there are variables where the values remain more or less like K^+ and CO_3^{2-}.




SO42-
700
600
500
400
300
200
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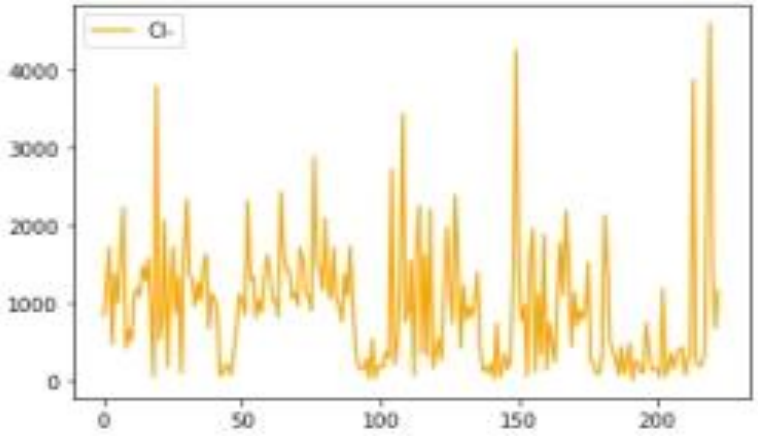
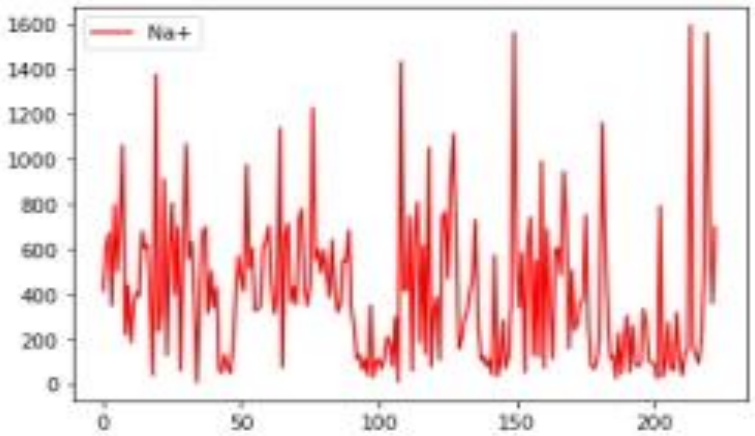
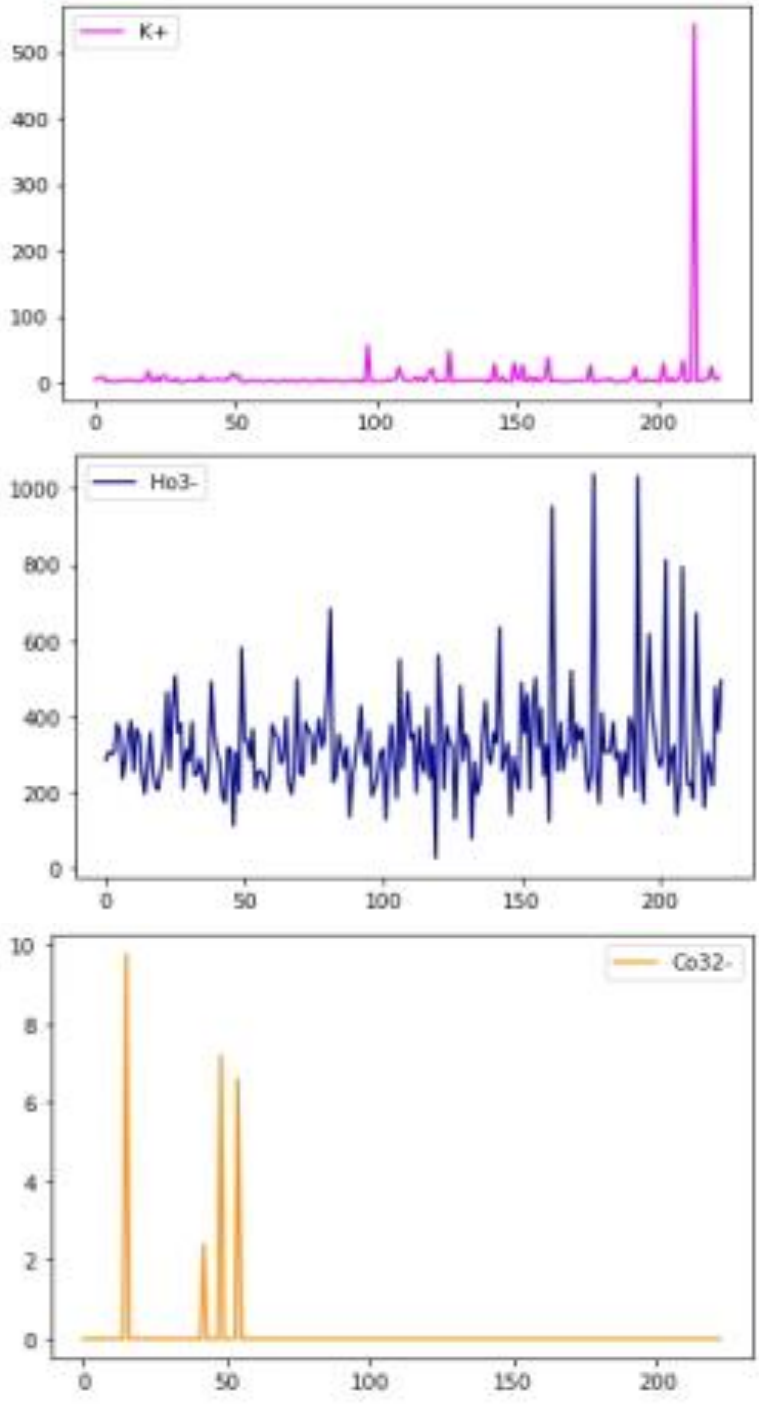
0
0
50
100
150
200


Cl-
4000
3000
2000
1000
0
0
50
100
150
200


Na+
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
200


K+
500
400
300
200
100
0
0
50
100
150
200
Ho3-
1000
800
600
400
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Co32-
10
8
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4
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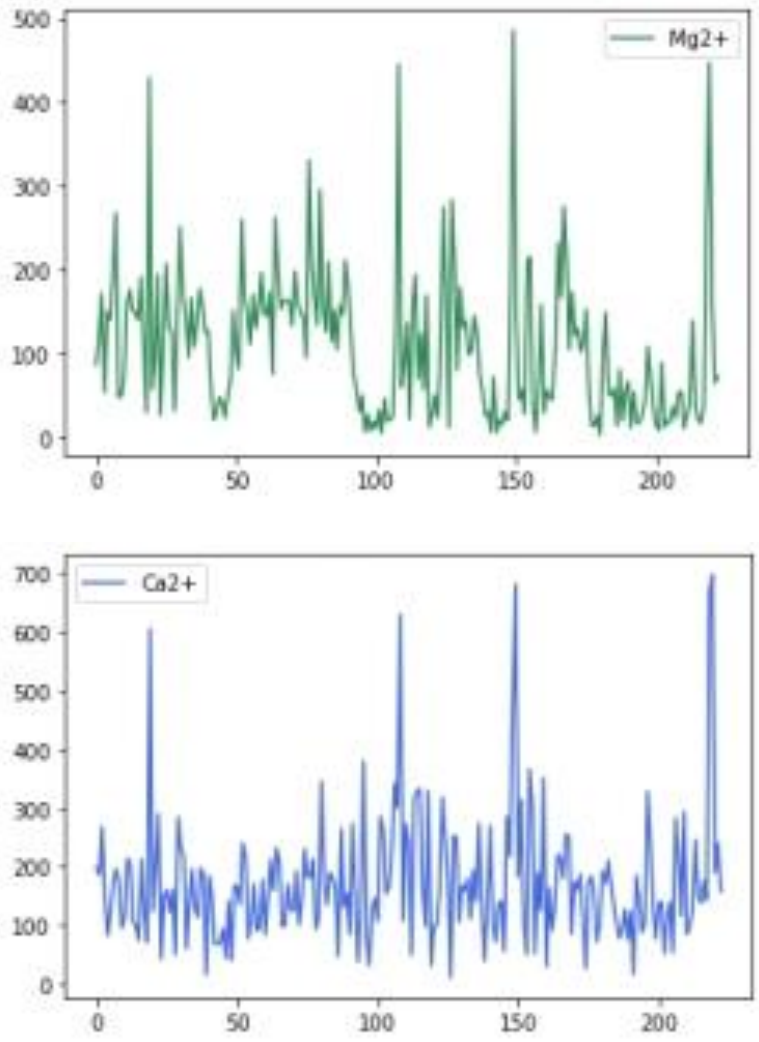

Figure 4. Plots of the raw data related to ten parameters
(X line represents the identification of samples while Y line represents the parameters)

The boxplot of the variables (Fig. 5) presents the evolution of the different variables, focusing mainly on the first quartile, the third quartile, etc. According to the obtained boxplot, 25% of the conductivities are between 0 and below 2000. However, the other values exceed 2000. pH and CO_3^{2-}, on the other hand, take the lowest values among all the variables.

In a third step, we made a general description of the variables using the following characteristics (Table 1): minimum, maximum, first quartile, median, average, third quartile, and maximum. The variables with the lowest minimum values are CO_3^{2-} and pH, and the highest maximum values are Ca^{2+} and Cl^-.

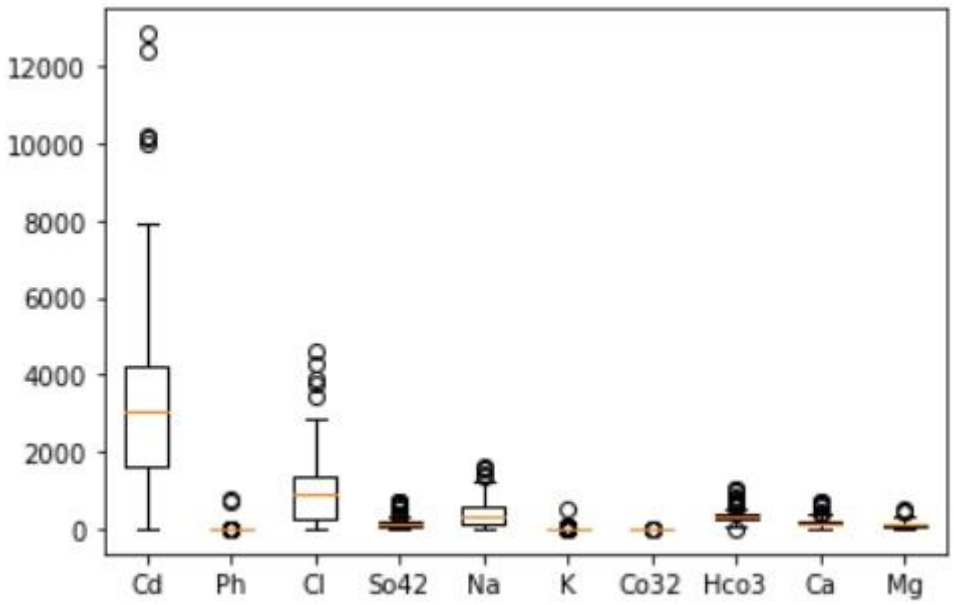

Figure 5. Boxplots of the used variables in the database.

Table 1. Descriptive statistical characteristics of the used variables.

	CE	pH	Cl^-	SO_4^{2+}	Na^+	K^+	CO_3^{2}-	HCO_3^-	Ca^+	Mg^{2+}
Min	7.54	0.2	12	11	5	0.29	0	26	11.2	3.68
1st Quartile	1595	7.138	276.2	64.67	124	1.157	0	244	102.8	37.83
Median	3014	7.3	867	117.5	349	2.640	0	305	154	101
Mean	3193	7.32	947.3	140.11	406.2	7.191	0.1159	323.3	171.6	109.07
3rd Quartile	4240	7.6	1352.8	174.75	604	4.030	0	363.8	212.0	155.5
Max	12835	8.7	4603	723	1595	542	9.76	1037	697	486

Step 3: Training and model validation process. In Machine Learning, and to prove the performance and reliability of an ML model, the database is divided into two main parts: The first part is dedicated to creating the ML model and the second part is dedicated to validating the model. Generally, the percentage of data dedicated to validation is between 20% and 30%.

Metric evaluation models. Model performance is evaluated using three statistical criteria: correlation coefficient (Asuero *et al*., 2006), root mean square error (RMSE) (Wang and Lu, 2018) and accuracy (Yin *et al*., 2019).

Correlation coefficient formulas are used to determine the strength of a relationship between data. The formulas return a value between -1 and 1, where: 1 indicates a strong positive relationship, -1 indicates a strong negative relationship. A result of zero indicates that there is absolutely no relationship whatsoever (Eq. (2)):

$$r = \frac{\sum_{i=1}^{n}(X_i - \overline{X})(Y_i - \overline{Y})}{\sqrt{\sum (X_i - \overline{X})^2 (Y_i - \overline{Y})^2}} \qquad \text{Eq. (2)}$$

RMSE is the square root of the variance of the residual errors (Eq. (3)). The lower value of the RMSE compared to the output ranges indicates a better fit of the model.

$$RSME = \sqrt{\frac{\sum_{i=1}^{N}(Predicted_i - Actual_i)^2}{N}} \qquad \text{Eq. (3)}$$

Machine learning model accuracy is used to determine which model best identifies relationships and patterns between variables in a dataset based on the input or training data. Accuracy is defined as follows (Eq. (4)):

$$Accuracy = \frac{Correct\ predictions}{All\ predictions} \qquad \text{Eq. (4)}$$

RESULTS AND DISCUSSION

Exploratory data analysis

A correlation matrix and evaluation of the input variables' significance were performed for further exploration of the variable. Table 2 shows the matrix correlation. Figure 8 highlights the correlation plot. Our purpose is to predict the conductivity as a function of the other parameters: a complete description of the different parameters involved in the study.

The results revealed that the conductivity (CE) has a strong correlation with the parameters Cl^- (0.92), Na^+ (0.91) and Mg^{2+} (0.87) and a moderate correlation with the parameters SO_4^{2-} (0.58) and Ca^{2+} (0.6). However, the conductivity correlates poorly with the parameters pH (0.06), K^+ (0.26), CO_3^{2-} (-0.008) and HCO_3^- (0.17). These results indicate that the variables Cl^-, Na^+, Mg^{2+}, SO_4^{2-} and Ca^{2+} better predict the conductivity. Although the other parameters are less correlated with the conductivity, they are needed to improve the prediction accuracy of the ML models.

Table 2. Correlation matrix of the used variables

	CE	pH	Cl^-	SO_4^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	Ca^+	Mg^{2+}
CE	1	0.0606	0.925	0.583	0.919	0.26	-0.008	0.17	0.607	0.87
pH	0.06	1	0.062	-0.038	0.111	-0.017	-0.013	0.039	-0.071	0.05
Cl^-	0.92	0.062	1	0.507	0.908	0.27	0.013	0.084	0.616	0.89
SO_4^{2+}	0.583	-0.03	0.507	1	0.541	0.024	-0.049	0.076	0.616	0.55
Na^+	0.9190.111	0.111	0.908	0.541	1	0.293	0.035	0.185	0.475	0.797
K^+	0.261	-0.0179	0.27	0.024	0.293	1	-0.015	0.232	0.071	0.034
CO_3^{2-}	-0.008	-0.0137	0.0135	-0.049	0.035	-0.0156	1	-0.0899	-0.122	0.004
HCO_3^-	0.17	0.00396	0.0842	0.076	0.185	0.232	-0.089	1	0.094	0.052
Ca^+	0.607	-0.071	0.616	0.6168	0.475	0.071	-0.122	0.094	1	0.608
Mg^{2+}	0.873	0.0523	0.89	0.554	0.797	0.0344	0.004	0.052	0.608	1

Simulated and real values. This part is dedicated to schematizing the simulated values created by the different models and comparing them to the real conductivity values in part dedicated to the validation. For example, starting with the Random Forest (Fig. 6), it turns out that a large percentage of the simulated values coincide with the actual values, except for a few points that deviate sharply and remarkably.

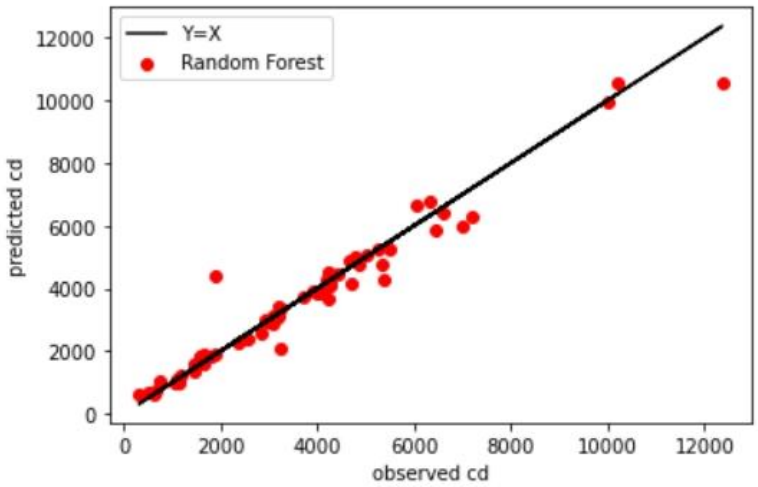

Figure 6. Scatter plots of the observed and simulated values for predicting the conductivity using the Random Forest Model

For the SVR model (Fig. 7), it is likewise found that several predicted values coincide approximately with real values, which shows a remarkable ability of the model to predict the conductivity in the groundwater. However, there are some locations where there is a considerable discrepancy between the predicted and actual values, especially in the last part of the plot.

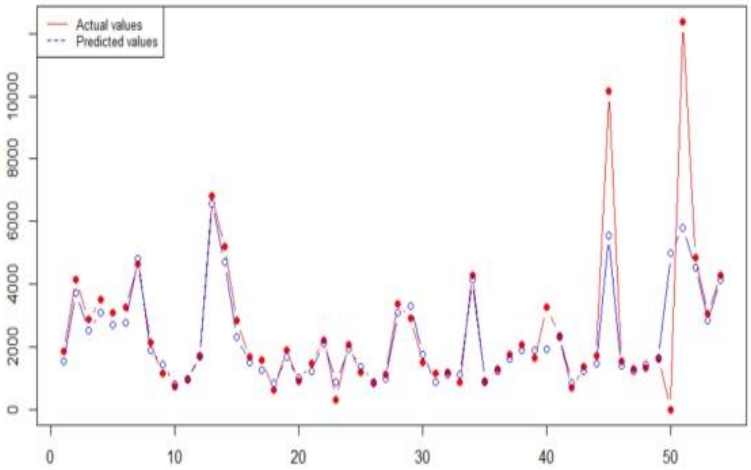

Figure 7. Scatter plots of the observed and simulated values for predicting the conductivity using the SVR Model

For the KNN model (Fig. 8), we notice that the points are more scattered compared to the model considering the RF. In addition, several points are far away from the actual values, namely the last points.

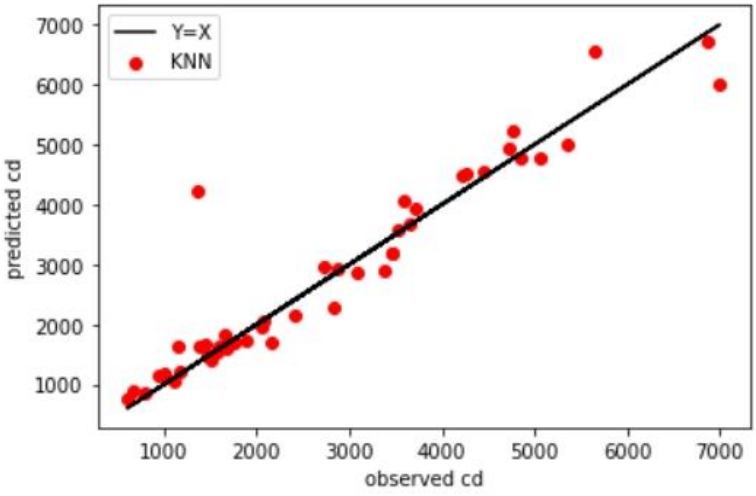

Figure 8. Scatter plots of the observed and simulated values for predicting the conductivity using the KNN Model

If we compare the three figures, we can see that the RF model is the model with the most overlap between the predicted and actual values. Most of the points rotate around the X=Y line, proving that this model can predict the conductivity with a high accuracy depending on the selected parameters.

General results

Before the training phase, the selected data were organized in a CSV file for processing. Thus, they were divided into two datasets: 372 samples were used for the training process and 93 samples were run in the validation phase. Then, the ML models were built in Spyder using the anaconda platform. In this sense, the evaluation metrics are measured by comparing the outputs of the validation part generated by the created model and the actual outputs. By comparing the two, we will be able to specify which model or algorithm performs better in terms of correlation, squared error and accuracy (Fig. 9).

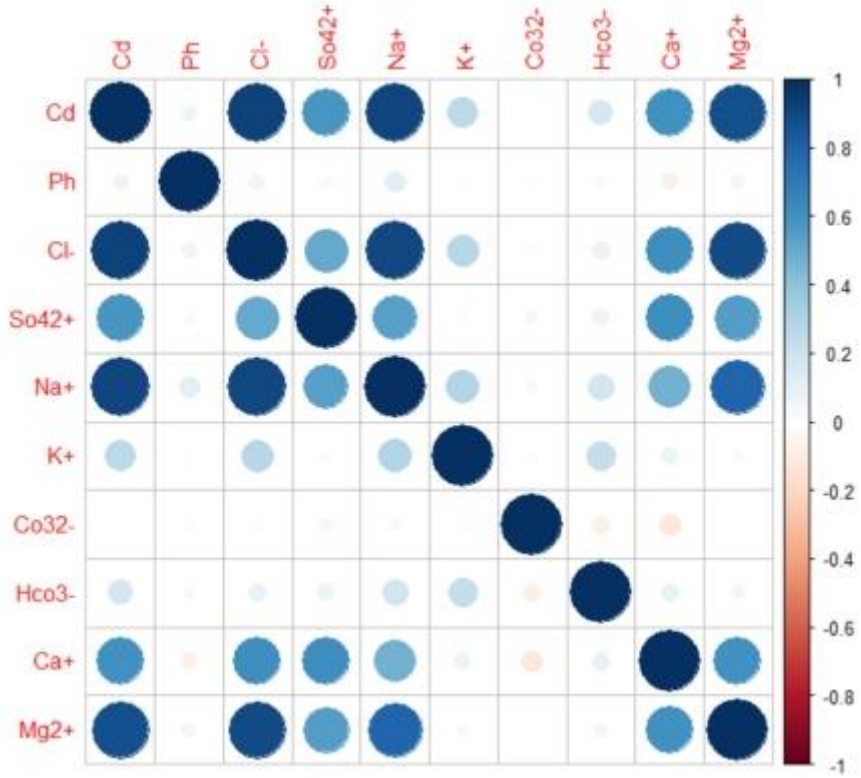

Fig. 9. The correlation plot of the used variables

Table 3 represents the RMSE, R-values and accuracy of the models during the training process. These results reveal that the RF model yields the best result in correlation r with a value of 0.966 compared to a value of 0.949 for the KNN algorithm and a value of 0.825 for the SVR model. For the value of RSME, we

calculated the normalized RMSE (Eq. (5)) using the following formula with y is the output parameter (Conductivity).

$$RMSEnormalized = \frac{RMSE}{y_{max} - y_{min}} \qquad Eq.(5)$$

The smaller the RMSE value, the lower the error rate and the ML model performs better. Through this indication, we can see that in terms of RMSE, the RF model performs better with an RMSE of 0.042 against an RMSE value of 0.082 for the KNN and a value of 0.102 for the SVR. Regarding accuracy, the RF model is the most accurate among the three models. The KNN algorithm provides an accuracy of 85.56% against accuracy of 83.86% for the SVR model.

Table 3. Evaluation metrics for RF, SVR and KNN models

Statistical indices	Random Forest (RF)	Support Vector Regression (SVR)	K-Nearest Neighbor (KNN)
Correlation r	0.966	0.825	0.949
RSME	0.042	0.102	0.082
Accuracy	89.19 %	83.86 %	85.56 %

Field practical implication in water management

Our study built and validated ML models to predict the conductivity using several parameters as predictors: pH, Cl^-, K^+, Na^+, etc. Following the results of this study, the ML models have proven to be accurate and robust in predicting conductivity (Table 3). In addition, we opted for several evaluation measures. These analyses reveal that the capabilities of the RF and KNN models are significantly higher than the SVR model in the present study. In terms of correlation and squared error and accuracy, the RF performs better. For the SVR, we notice a high mean square error of 0.102, which presents a difference of more than 50% between the RF and the SVR in terms of square error. The objective of this paper was to predict the conductivity level to assess the groundwater quality with the minimum costs.

To measure the conductivity of Water, it requires a whole measurement process and equipment such as a probe or a conductivity meter. The basic principle of conductivity measurement consists of several steps: the measuring instrument applies an electrical voltage to the solution to be measured. An electric current flows according to the conductivity. Depending on the method or application, either the meter applies a constant voltage. It records the change in electric current, or the meter applies a constant current and evaluates the voltage change. In addition to the measuring equipment costs, there is also the necessity to maintain the equipment. Indeed, wear and tear and dirt reduce its reliability and introduce measurement errors. A regular calibration allows detecting of unreliable components. It restores them to their normal state, for example, by cleaning them. Non-maintenance can therefore cause divergent measurements

and thus a dispersion of the measurements. Cleaning is also a possible cost of measuring the conductivity of Water. Cleaning can be achieved with hot Water or vinegar. All this leads to a set of costs and expenses that is summarized in the following equation (Eq(6)):

$$General\ cost = Equipement\ cost + Maintaining\ cost + Cleaning\ cost + Other\ possible\ costs \qquad Eq.\ (6)$$

Equation 5 highlights the high cost of measuring the conductivity degree. Our study, leveraging Machine Learning models, allows us to reduce these costs distinctively, with high accuracy and performance.

CONCLUSION

In this paper, our objective was to predict the conductivity contributing in the evaluation of grounded water quality as a function of several parameters such as: Mg2+, Na+, Cl-... In this perspective, we used several ML models using several input parameters. Also, we performed a statistical description that calculates the correlation matrix and the different measurements of each variable. Therefore, Random Forest, SVR and KNN models were developed and evaluated to predict the conductivity using several input parameters. This study leads to several results:

ML models based on several input parameters are All three models (RF, SVR and KNN) produced a correlation more significant than 80%.

All three models (RF, SVR and KNN) give more than 80% accuracy.

The RF model generates more significant results compared to the other models.

The ML models allowed us to predict the conductivity with high accuracy and low error as a function of several other parameters.

The models created to minimize the costs of measuring conductivity with special equipment are highly effective tools. Therefore, they should be recommended to predict water quality parameters. These models will improve groundwater quality monitoring for irrigation purposes in real-time and at a low cost.

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MEDICINAL PROPERTIES AND MAIN INDICATORS OF SEED AND OIL QUALITY OF FLAXSEED - *Linum usitatissimum* L.

SUMMARY

Linum usitatissimum L. contains up to 45% oil. Linseed oil is a favourable fatty acid composition with a high linolenic acid content. Flaxseed oil is a valuable raw material for food and medical purposes due to its fatty acid composition and high content of linolenic acid. Omega-3 polyunsaturated fatty acids (PUFA) have shown that these compounds have therapeutic potential in several indications in neurology, psychiatry and cardiovascular disease. The aim of this study was to assess the quality of flax seed oils extracted from flax seed produced under different environmental conditions. Flax seed quality is highly dependent on weather conditions in the year of flax production, therefore the influence of the extreme weather events to the seed quality traits were also examined. The material consisted of nine samples of cold extracted oil from three flax varieties. Flaxseed oil production took place at five locations from 100 m to 700 m above sea level. The results indicate that high quality flax seed oil production is conditioned with proper farm technology, weather conditions and adequate storage. Oil rancidity and self - ignition of seed appeared in a case of improper seed storage. The expansion of the oilseed flax production should be accompanied by the education of farmers and potential consumers of flax products.

Keywords: *Linum usitatissimum* L., flaxseed oil, seed, sensory traits, chemical traits, medicinal properties

INTRODUCTION

Linum usitatissimum L. contains up to 45% oil. he fatty acid composition, especially the high linolenic acid content of linseed oil makes it a valuable raw material for food and medicinal purposes. Omega-3 polyunsaturated fatty acids (PUFA) have shown that these compounds have therapeutic potential in several indications in neurology, psychiatry and cardiovascular disease. Fiber flax had

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been a common crop throughout the Balkans until the introduction of man-made fiber. In some regions, local flax varieties were used for oil production too. Flaxseed contains 26–45% oil (Diedrichsen, 2001; Popović *et al.*, 2017, 2019a; 2021; Ikanović *et al.*, 2020; Ikanović and Popović, 2020). Approximately 22% of the oil is located in the seed coat and 4% in the embryo. The oil ispresent mainly as triacylglycerols in oil bodies having an average diameter of 1.3 μm (Daun *et al.*, 2003). Approximately 70% of all the linseed oil produced worldwide is destined for technical applications and 30% is for food production (Järvenpää, 2000). Flaxseed oil is used in a wide variety of applications, including additives in PVC plastics, antirust agents, laquers and paints, aroma substances for the food industry (Bonnarme *et al.*, 1997) or volatile com-pounds for obtaining a fresh green odour to off setthe decreased odour caused by the processing of vegetables (Noordermeer *et al.*, 2002). The lack of interest in this plant species had lasted until the end of the past century, so most of local varieties were irretrievably lost. The international conference "Bast Fibrous Plants Today and Tomorrow" held in St. Petersburg (September 1998) was a key event of importance for the return of flax to the Balkans, this time oil seed varieties (Kondić and Nožinić, 1998; Filipović *et al.*, 2013; 2021). In the following years, flax varieties from the gene bank "N.I. Vavilov" have been tested in the Institute's experimental field in Banja Luka. Commercial oil seed flax production (syn. linseed) has been developing after the Third Global Work shop of the FAO/ESCORENA European Cooperative Research Network on Flax and Other Bast Plants "*Bast Fibrous Plants for Healthy Life*" held in Banja Luka in 2004. The Institute for Natural Fibers from Poznan (director prof. Richard Kozlowski) and the Institute in Banja Luka organized this event in very sucesfull way. As local flax varieties had been lost, the first activities were focused on the introduction of flax seed from the Agricultural Institute in Zajačar in Serbia (Garić and Mandić, 2004). Some mountain regions (Petrovačko polje, Manjača) were recognized to be suitable for "ecologically friendly" and organic oil seed flax production (Nožinić, 2009; Nožinić *et al.*, 2012; 2013; 2016). The ecological advantages of mountain regions are due to the absence of invasive weeds as *Ambrosia artemisiifolia* and unpolluted soils (some plots have not been cultivated for 30 years). The production of cold extracted vegetable oils at the Institute began in 2011. The transfer of knowledge from the Institute to the farmers resulted in about 30 oil mills, which have been contributed to farms' incomes. Flax seed, flax seed ingredients, especially flax seed oil show evident functional food effects what has been proved in many research studies (Oomah and Mazza, 1998; Oomah, 2001; Mani *et al.*, 2011; Rodriguez *et al.*, 2013; Caligiuri *et al.*, 2014; Glamočlija *et al.*, 2015; Caligiuri *et al.*, 2016; Parikh *et al.*, 2018). For this reason flax seed products reach high price on the local market. However, as flax has a shallow root system, its production in the lowlands is more and more difficult for frequent droughts and global warming. Extremely high temperatures can affect seed and oil quality even before harvesting. Unlike lowlands, some mountain valleys with moderate summer temperatures, high number of sunny hours and permanent wind

activity create almost ideal conditions in the period of oil seed flax ripening. Flax requires certain storage conditions. Banal mistakes, such as storing seeds in a thick layer in a plastic package can lead to product spoilage or even self-ignition. So, it is not easy to produce quality flax seed oil and earn desired money. The aim of this paper was to describe the quality of flax seed oil samples extracted from flax seed produced in different agroecological conditions.

MATERIAL AND METHODS

Analysed material consisted of nine samples of cold extracted oil from three flax seed varieties. The first oil sample (BL-Z) was extracted from yellow seed variety Zlatko (eng. "Golden") produced at the Institute's experimental field in Banja Luka. The second oil sample (BL-O) was extracted from Romanian variety Olin (brown flax seed) harvested on the same location. Other oil samples (Table 1) originate from one local flax variety. Its origin and classification (oil or fibre form) are not clear. This seed had been stored on the old house roof in the village Vrtoče for many years, then sown in 2004. Since 2004, the seed has been multiplied on more locations with different climate conditions showing excellent adaptability. The oil sample (OR) originated from Orašje, a fertile lowland area besides the River Sava (Table 1). The organoleptic characteristics of flax seed and flax seed oil were evaluated on the basis of the visual observations, odor and taste. The flaxseed diseases were determined in the Laboratory for phytopathology at the Agricultural institute of Republic Srpska (abbr. Institute). The oil was extracted in the Institute's oil mill. Chemical analysis of oil included Peroxide value (PV), free fatty acids (FFA) and content of fatty acids. As flax seed quality is highly dependent on weather conditions in the year of flax production, the influence of the extreme weather events to the seed quality traits were commented too.

Table 1. Basic data of flaxseed production locations

Location	Longitude	Latitude	Altitude (m)	Relief form
Banja Luka	17.2200	44.7800	About 150	River valley
Vedro polje	16.3896	44.5064	650 - 700	Mountain valley without river
Vrtoče	16.1753	44.6347	About 600	Mountain valley without river
Derventa	17.9067	44.9792	150 - 200	Gentle slopes
Orašje	18.6935	45.0368	About 100	Large river valley

Other oil samples were extracted from flaxseed produced in the region of the Petrovačko polje (abbr. PE). The abbreviation (PE-VP) is related to the seed produced in the valley Vedro Polje (eng. "Clear Field"), which is a part of Petrovačko polje (PE). The name of this valley corresponds to its main climatic feature (high number of sunny hours). The abbreviation PE-V is related with the village Vrtoče in the central part of Petrovačko Polje. The spreading of local variety began by the enthusiast Miodrag Latinović from that village. The

organoleptic flax seed properties were evaluated on the basis of the visual flax seed characteristics (smooth or wrinkled seed surface, seed gloss, seed size, visible traces of pathogens or pathogen activity on seeds), odor and taste. The diseases were determined in the Institute's Laboratory for phytopathology at the Agricultural institute of Republic Srpska (abbr. Institute). The oil was extracted in the Institute's oil mill. Chemical analyses of oil included Peroxide value (PV), free fatty acids (FFA) and content of fatty acids, have been done by the Laboratory of "Bimal Group" in Brčko. Peroxide value (PV) was determined by method ISO 3960, free fatty acids (FFA) were determined according to ISO 660 and content of fatty acids by GC methodology (ISO 5508, 5509). The influence of the extreme weather events to the seed quality traits was described on the basis of climatic data from the meteorological stations Banja Luka and Drinić - Petrovac.

The result of soil sample from Vedro Polje is quite representative for other locations on Petrovačko Polje (Table 2). These soils are shallow and the depth depends on the position in the mountain field. The content of humus and potassium is high while available phosphors is in deficit. Thanks to dolomite stone under the soil, its reaction is neutral. The alluvial soil in the valley of the river Vrbas (experimental field of the Agricultural Institute has relative good fertility while physical traits are upset with intensive irrigation of previous crop (hybrid maize). The alluvial soil in Orašje belongs to the category of deep alluvial soils with high fertility.

Table 2. Soil fertility traits at flax plots

Location/trait	Year	Depth in cm	pH in H_2O	pH in KCl	Humus (%)	P_2O_5 mg/ 100 g	K_2O mg/ 100 g
Banja Luka	2009	0-25	7.2	6.6	3.1	16.7	23.0
Vedro polje	2014	0-25	7.5	6.7	7.0	2.0	47.0
Derventa	2013	0-25	5.8	5.3	2.1	1.1	13.4
Orašje	2013	0-25	6.4	5.8	4.3	13.8	20.0

Table 3. Total precipitation (P, lit./m^2) and maximum temperatures (T, ˚C) in Banja Luka (150 m alt.) and Drinić (730 m alt.) in the flax vegetation seasons

Year/month	April		May		June		July		August	
	P	T	P	T	P	T	P	T	P	T
BL, 2009	40	26.4	49	34.1	153	35.7	43	38.1	138	37.4
BL, 2013	63	31.8	120	31.7	54	36.0	27	41.6	36	41.1
BL, 2014	214	25.8	217	31.2	97	34.1	139	34.3	177	34.1
DR, 2014	221	22.0	217	29.4	157	31.8	174	32.4	177	32.6
BL, 2018	20	31.0	137	31.1	103	34.5	84	34.0	82	35.3
DR, 2018	16	25.3	122	26.5	81	29.0	28	29.2	106	31.5
BL, 2019	105	29.9	225	27.2	123	35.3	59	36.8	49	37.7
BL, 2020	27	28.5	104	30.0	62	35.1	72	35.7	142	36.7
BL, 2021	69	27.8	82	31.1	12	39.3	63	40.2	57	-

Abbrivitatons: BL - Banja Luka, DR - Drinić

The influence of the extreme weather events to the seed quality traits was described on the basis of climatic data from the meteorological stations Banja Luka (150 m alt.) and Drinić at the edge of Petrovačko polje (700 m alt.), Table 3. Though Derventa and Prnjavor do not have meteorological stations, the temperature regime from the station Banja Luka is quite representative for these production regions too. The meteorological data for the station Drinić have not been available for all vegetation seasons. Comparable data were available for 2014 and 2018.

RESULTS AND DISCUSSION

Flax seed production parameters

Proper agrotechnique from sowing till harvesting is the first precondition for high quality flaxseed. Because of the weak root system and specific ecological requirements in the ripening period, oilseed flax belongs to the crops which are very vulnerable to the extreme weather conditions (Popović *et al.*, 2017; 2021, 2022a; b). The quality of flax seed in 2013 was highly dependent on harvest date. Better seed quality was obtained when the harvest was done till the mid of July. The flax seed from Orašje (OR), which was harvested on July 12 provided oil with excellent taste as well as desirable PV and FFA percentage (Table 4, Figure 1). The flaxseed that was maturing in the second part of July and in the first part of August was exposed to extreme heat. In the third decade of July 2013, the lowland regions experienced maximum temperatures about 40˚C (Banja Luka, 41.6˚C).

Table 4. Results of flaxseed oil analyses, g*

Sample	Harvest	Analysed	PV	FFA	C18:3	C18:2	C18:1	C18:0	C16:0
BL-Z	2009	IV, 2010	0.31	0.69	56.0	15.2	18.0	3.6	5.2
BL-O	2009	IV, 2010	0.21	0.51	54.2	12.6	20.6	4.4	5.8
OR	2013	IX, 2013	1.74	0.21	50.3	12.4	25.5	4.4	6.1
PE-V	2014	III, 2015	2.99	0.29	61.7	14.7	15.3	3.6	4.5
PE-V	2019	XII,2019	0.00	0.36	59.6	12.9	17.6	3.5	5.5
PE-VP	2020	I, 2021	0.66	0.64	60.1	12.3	18.9	3.6	4.7
PE-VP	2019	I, 2021	0.34	0.43	-	-	-	-	-
PE-VP	2018	I, 2021	10.46	3.20					
DE	2020	III, 2021	15.03	4.11	53.2	12.9	18.1	3.5	4.9

*PV-Peroxide value; FFA-free fatty acids; C16:0-palmitic acid; C18:0-stearic acid; C18:1-oleic acid; C18:2-Linoleic acid; C18:3- alfa-linolenic acid, ALA

The year 2014 can rightly be called the "Black year" for oilseed flax production. Heavy rains in the period April - September (Banja Luka, 944 lit/m^2; Drinić - Petrovac, 1.246 lit/m^2) led to the development of serious flax diseases (flax wilt), which caused the decay of plants in the period of emergence as well as rapid reduction of flaxseed yield and quality. The unprecedented flood in April 2014 strangled the flax in the varietal trial at the Institute's experimental field in Banja Luka. Small quantity of flax seed harvested in 2014 had significantly lower germination in 2015. Flax survived stress conditions in 2014 just on a few plots

with permeable soils in the mountain region of Petrovačko polje. The oil from one sample of the flaxseed produced in 2014 was analysed in 2015. Although the content of PV and FFA indicated relative good oil quality (tab. 4). Almost all oil seed flax fields in 2014 were infested by *Fusarium oxysporum*, which is a common fungi in the humid conditions. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. lini (Fol), can infect flax at any growth stage and may result in 100% disease incidence in certain cultivars (Panjan, 1968; Kommedahl *et al.*, 1970). The pathogen which can be seed-borne or soil-borne, invades through roots and develops in the xylem. Radman (1978) states that this pathogen makes more damage on oilseed flax varieties than fiber ones. Very high content of linolenic fatty acid (omega - 3) in the oil from the seed harvested in 2014 might be the result of extremely long vegetation. The longer vegetation, the higher the content of polyunsaturated fatty acids in oil crops (Kastori, 1991).

Perfect flaxseed oil was produced from the flaxseed harvested in the village Vrtoče (PE-V) in 2019 (Table 4). Total absence of the reactive oxygen in this oil sample indicates proper production technology and proper oil storage. Thanks to proper field production technology and absence of invasive weeds (*Ambrosia sp.*), there is no need for herbicide treatment. It is an organic model of production, without certification. As all activities "from the field to the oil" take place under full farmer's control, all production risks are reduced to minimum.

Oil samples from “Vedro polje” (eng. Clear Field) had excellent quality except for 2018. The name of the valley indicates desirable environmental condition for flax seed oil production.

Flaxseed oil has the highest needs in water during the phases of the intensive growth and flowering in May and June. Since June 2021 was the driest one (Banja Luka 12 lit/m^2) in the period of measurements (Banja Luka, since 1881), shallow root system of flax could not provide enough water for normal flax growth and pollination. Moderately warm and sunny weather in July stimulates oil synthesis and ripening of flax seed. July 2021 brought hellish heat (Banja Luka, max. 40.2˚C), which caused shrinking of flaxseed and negligible yield. Evident is negative effects of extreme weather conditions to flax production.

Improper flaxseed storage can cause spoilage and great damage. The oil from improperly stored seed in region of Derventa (DE) had a bitter taste, too high PV and FFA percentage (Table 4). Rancid oil can be used for natural protection and decoration of woody surfaces.

Compared with the lowlands, mountain region in “Petrovačko polje”, he had more sunny hours and less foggy days. Rich mountain relief and closeness of the Adriatic Sea create permanent winds' activity. The more air circulation, the less plant diseases appeare. Unlike lowlands, the temperatures in the mountain valleys rarely exceed 30˚C. Such moderate temperatures favor longer oil synthesis providing oil with a very high content of polyunsaturated fatty acids (omega-3). The fact is that “Petrovačko polje” meets all conditions for the flax seed products with geographical origin.

Two oil samples (BL-Z, BL-O) from the seed harvested at the Institute's experimental field in Banja Luka in 2009 had excellent taste, low PV and FFA percentage, which indicated proper seed and oil management. That year was relatively suitable for oilseed flax production. Typical June with high rainfall (Banja Luka, 153 l/m^2) provided satisfactory conditions for normal growth, pollination and seed development. Though frequent rains caused occurence of new flowers for longer periods (partial retrovegetation), dry and hot weather in July favored uniform flaxseed ripening. The Serbian variety Zlatko was released in 2003 in the Agricultural Institute in Zaječar (Stanković *et al.*, 2003), then tested in Banja Luka region in the period 2006-2014. Unfortunately, this valuable variety was lost during the flood in 2014. Romanian variety Olin provided excellent oil yields in the mountain region of Manjača (village Sitnica). This oil had a very "soft" taste, acceptable for consumers.

Having in mind that domestic oilseed flax production is limited on one old variety, it is time for new genetics. New oilseed flax varieties, NS Primus and NS Marko, selected in the Institute of Field and Vegetable Crops in Novi Sad obtain extremely stable and good yields, have good adaptability and high nutritional value (Popović *et al*., 2017, 2021, 2022b).

Some medicinal effects of flaxseed ingredients

All ingredients of flaxseed (oil, protein, dietary fibers, phytoestrogens, mucilage, vitamins) offer health benefits, so can be used in medicinal purposes like functional foods or pharmaceutical products (Popović *et al.*, 2017, 2019a; 2019b; 2021; 2022). Flaxseed contains 26–45% oil (Diedrichsen, 2001; Popović *et al*., 2017, 2019a; 2021; Ikanović *et al*., 2020; Ikanović and Popović, 2020). The oil is present mainly in oil bodies having an average diameter of 1.3 μm (Daun *et al*., 2003). Flaxseed (syn. linseed) is one of the richest plant sources of the ω-3 fatty acid alpha-linolenic acid (ALA, C18:3 ω-3), vitamins of groups A, B and E and used in the food and pharmaceutical industry (Popović *et al.*, 2017, 2019a; 2019b; 2021; 2022b), Figure 1.

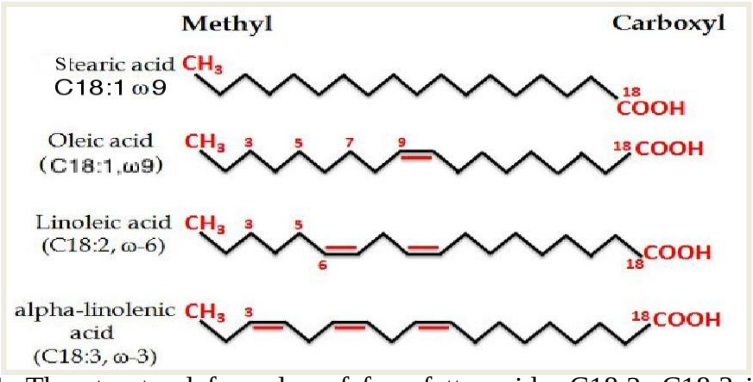

Figure 1. The structural formulae of four fatty acids. C18:2; C18:3 is dietary essential fatty acids (Popović *et al*., 2019a; 2022b)

Flaxseed oil is the richest herbal source of alpha-linolenic acid (ALA) what is of great importance for preventing the occurrence of cardiovascular disease (CVD) with nutritional interventions. The increased use of omega-3 fatty acids can be a powerful example of nutritional strategy that may produce significant cardiovascular benefits. Because of its high ALA content, the use of flax seed has been advocated to combat CVD (Rodriguez-Leyva *et al*., 2010).

The author states that sixty patients of type 2 diabetes were fed a daily diet for 3 months, with flax seed gum was incorporated in wheat flour chapattis, along with six wheat flour chapattis containing flax seed gum (5g), as per recommendations of the American Diabetic Association. Biochemical blood profiles in the control group, which consumed an identical diet but chapatis were without chewing gum, showed that fasting blood sugar in the experimental group decreased from 154 ± 8 mg/dl to 136 ± 7 mg/dl (P= 0.03) and total cholesterol from 182 ± 11 mg/dl to 163 ± 9 mg/dl (P=0.03). Results showed a decrease in low-density lipoprotein cholesterol from 110 ± 8 mg/dl to 92 ± 9 mg/dl (P=0.02) and the efficacy of flax gum in the blood biochemistry profiles of type 2 diabetes (Thakur *et al.*, 2009).

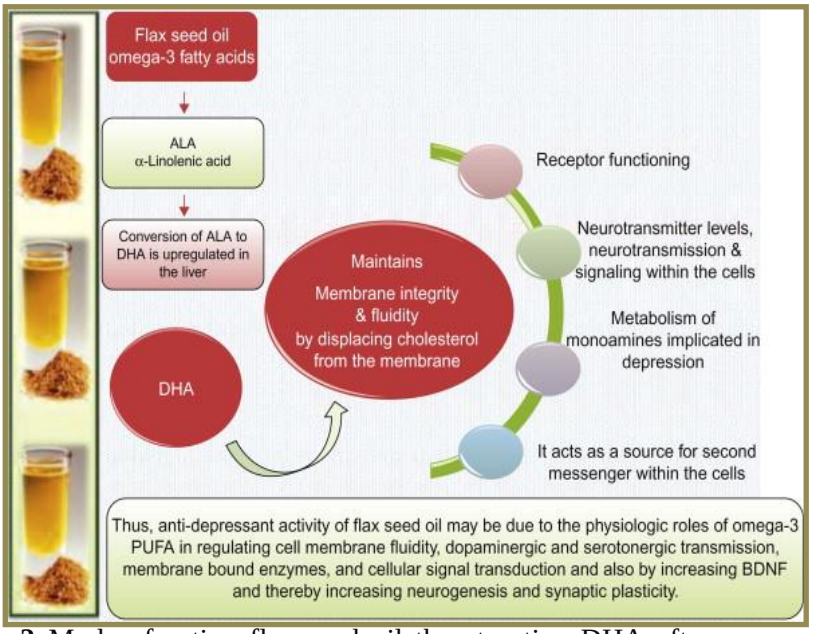

Figure 2. Mode of action flax seed oil throut action DHA after conversion of ALA and potential health effects (Chandola & Tanna, 2014; Popović *et al*., 2022b)

Reducing and preventing the occurrence of cardiovascular disease (CVD) through nutritional interventions is a therapeutic strategy with increased use of omega (ω)-3 fatty acids that can produce significant cardiovascular benefits. Flaxseed is one of the richest sources of the plant-based ω-3 fatty acid, alpha-

linolenic acid (ALA). Based on the results of clinical trials, epidemiological investigations and experimental studies, ingestion of ALA has been suggested to have a positive impact on CVD. Because of its high ALA content, the use of flax seed has been advocated to combat CVD (Chandola and Tanna, 2014; Calado *et al.*, 2018).

Flax seed is a rich sourse of ALA which is endogenously converted into DHA. DHA, by mantaining membrane fluicidi, by displacing holesterol from the membrane, corrects receptor functioning, regulates neurotransmiter levels, neurotransmision, and singnaling within the cells. Its acs as a second messenger sourse with cells (https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/linseed-oil), Figure 2. It helps in metabolism of monoamines implicated (Derman *et al.*, 2005; Watson, 2014) etiopathogenesis of depresium. Many studies with omega-3 polyunsaturated fatty acids (PUFA) indicate that these compounds have therapeutic potential in neurology and psychiatry. Omega-3 PUFA leads to increased survival of neurons and glia, and improved neurological outcome. The DHA injection led to an increased neuronal and glial cell survival, and the effect of the DHA injection was amplified by addition of DHA to the diet (Michael-Titus, 2007). Flaxseed is rich in omega-3 fatty acids, α-linolenic acid, lignan, and fibers. Flaxseed lignans, 95% are made of the predominant secoisolariciresinol diglucoside (SDG), which is converted into enterolactone and enterodiol, both structurally similar to estrogen, they can bind to cell receptors, decreasing cell growth (Figure 3). Results have shown mixed findings, and much more human research is needed. Intake of omega-3 fatty acids is related to the reduction of breast cancer risk, prostate and colon (Calado *et al.*, 2018; Popović *et al.*, 2019a; 2021).

Figure 3. Flaxseed oil and healt benefit; https://www.lybrate.com/topic/benefits-of-flax-seed-oil-and-its-side-effects; Popović *et al.*, 2022b.

Treatment with omega-3 PUFA could represent a promising therapeutic approach in the management of neurological injury. Bifidobacterially produced C18:3 CFA may have potential in the control of colon cancer (Hennessy *et al.*, 2016). The therapeutic potential in the gastrointestinal tract is indicated by their inhibitory properties against common gastrointestinal pathogens (especially methicillin-resistant *S. aureus* ATCC 43300).

CONCLUSION

Flax seed oil quality is highly dependent on proper technology production, locality and environmental conditions. Evident is negative effects of extreme weather conditions to flax production. Oil seed flax has the highest needs in water during the phases of the intensive growth and flowering in May and June. Mountain region in "Petrovačko polje" provides better agro ecological conditions for oil seed flax production than low lands. Rich mountain relief and closeness of the Adriatic Sea create permanent winds' activity. He had more sunny hours and less foggy days compared with the lowlands.Unlike lowlands, the temperatures in the mountain valleys rarely exceed 30˚C. Moderately warm and sunny weather in July stimulates oil synthesis and ripening of flax seed. Such moderate temperatures favor longer oil synthesis providing oil with a very high content of polyunsaturated fatty acids (omega-3). "Petrovačko polje" meets all conditions for the flax seed products with geographical origin.

All ingredients of flaxseed (oil, protein, dietary fibers, phytoestrogens, mucilage, vitamins) offer health benefits, so can be used in medicinal purposes like functional foods or pharmaceutical products. The increased use of omega-3 fatty acids can be a powerful example of nutritional strategy that have therapeutic potential in neurology and psychiatry, may produce significant cardiovascular benefits and cancer prevention.

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Aleksandar VEMIĆ [1*]

THE MOST IMPORTANT FUNGI ON WYCH ELM (*ULMUS GLABRA*) TREES IN MONTENEGRO

SUMMARY

This paper contains results of investigation of the most important fungi found on wych elm (*Ulmus glabra*) trees in Montenegro. During the monitoring, 21 fungal species were found on wych elm (*Ulmus glabra*) in Montenegro. Fungus *Ophiostoma novo-ulmi* was the most frequent on young trees while species *Fomes fomentarius* and *Daldinia concentrica* were the most frequent on old trees. Fungi *Mycosphaerella ulmi* and *Stegophora ulmea* were the most common species on leaves. Majority of fungi in this study was for the first time recorded on wych elm (*Ulmus glabra*) in Montenegro. Obtained results will contribute to knowing about fungi associated with Dutch elm disease as well as fungi colonizing healthy trees left to spontaneous growth.

Keywords: *Ulmus glabra*, fungi, occurrence, distribution, trees decline

INTRODUCTION

Elm genus contains about 35 species of trees and shrubs (Cvjetićanin *et al*. 2016). In domestic forests, wych elm (*Ulmus glabra* Huds.) trees represent autochthonal species and often occur as admixed species in beech (*Fagus sylvatica*) forests (Tomić, 2004; Cvjetićanin *et al*., 2016).

Species from elm (*Ulmus* spp.) genus have great ecological importance that is even bigger because they are endangered across areal by Dutch elm disease. Rare localities like "Biogradska gora" National Park have forests with fully developed trees that avoided decline due to Dutch elm disease. These localities are from especially interest in discovering pathogenic fungi that colonize trees more tolerant to Dutch elm disease.

Early investigations of fungal species on genus *Ulmus* across the world were relatively rare. In his publication Saccardo (1898) described above 50 fungal species on genus *Ulmus* worldwide. Later, (Groove, 1935; Groove, 1937) recorded nearly 30 fungal taxa within this genus. On western *Ulmus* trees, Ellis and Ellis (1985) described above 30 fungal species while Phillips and Burdekin (1992) listed about 17 fungal species. In summary, early researches about fungal

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diversity on wych elm (*Ulmus glabra*) were rare, and besides Saccardo (1898) none of above literature sources precisely described the diversity of fungi on specific tree species from genus *Ulmus*. Species wych elm (*Ulmus glabra*) is from especial importance as widely distributed in Europe and one of the most endangered from Dutch elm disease. Saccardo (1898) reported 15 fungal species and Lanier *et al*. (1976) reported 5 fungal species on wych elm (*Ulmus glabra*) trees. Brasier (1981) reported 6 main fungi causing cancer disease in wych elm (*Ulmus glabra*) worldwide and 1 species causing tar spots on leaves. Also, some of these fungi, respectively species *Phomopsis oblonga* (Desm.) Trav. has been proved to be antagonistic against Dutch elm disease (Webber and Gibbs, 1984).

Recent investigations of the most important fungi on wych elm (*Ulmus glabra*) were still relatively rare. Typical plant pathology literature sources (Horst, 2013) listed the same fungal species as early researches. New researches were primarily focused on endophytic species and associated with gall-making insects (Kowalski, 2004). However, Medarević *et al*., (2011) found 10 fungal species on single wych elm (*Ulmus glabra*) tree in Goč nature reserve in Serbia. Also, new studies of fungi on wych elm (*Ulmus glabra*) trees were focused on finding of highly pathogenic species like *Botryodiplodia hypodermia* (Ellis & Everh.) Buisman outside their natural range (Bartnik *et al*., 2018). Recent investigations about fungal species on wych elm (Ulmus glabra) reported *Ophiostoma novo-ulmi* Brasier in "Biogradska gora" National Park as locality previously without Dutch elm disease (Vemić, 2022).

Pathogens of wych elm (*Ulmus glabra*) in Montenegro are not much investigated. Knowledge about fungal species colonizing wych elm (*Ulmus glabra*) trees in this part of their areal can help in understanding patterns of decline before or after Dutch elm disease as main disease of elm trees. Also, knowledge about fungal pathogens of wych elm (*Ulmus glabra*) trees will help in screening trees that are more tolerant to Dutch elm disease and other pathogens at the same time for breeding programs. Another theoretical importance of these results is to study mycological complex on wych elm (*Ulmus glabra*) in this part of its areal.

Practical application of these results on global scale is to avoid production of plant material tolerant to Dutch elm disease but susceptible to other fungal pathogens. Also, on local scale practical application of obtained results is to prevent different fungal diseases in places where "disease escape" of *Ophiostoma novo-ulmi* is present or potentially produced seedlings tolerant to Dutch elm disease will be planted. Aims of this research were to investigate diversity of the most important fungi on wych elm and classify their role in decline progress through the number of first reports and distribution on different wych elm substrates in Montenegro. Tested null hypotheses were: a) Majority of recorded species aren't for the first time found on wych elm (*Ulmus glabra*) trees in Montenegro; b) Fungal species don't occur in succession on infected wych elm (*Ulmus glabra*) trees in Montenegro; c) There isn't difference in distribution of fungal damages on different organs of wych elm (*Ulmus glabra*) trees in

Montenegro. In forward, results about investigating the pathogenic fungal complex of wych elm (*Ulmus glabra*) trees in Montenegrin forests were showed. Some saprophytic fungal species also were showed because their succession was associated with investigated parasitic fungi.

MATERIAL AND METHODS

Field researches were performed in wide range of different localities in Montenegro, including all managed forests and National Parks "Durmitor" and "Biogradska Gora". Field research included monitoring of health condition of wych elm (*Ulmus glabra*) trees whereby the occurrence of symptoms and fruit bodies of fungi were observed. Monitoring was performed during the period of 2017-2019 years, three times in each year. Trees in all stages of development were examined.

Samples from symptomatic trees were collected for laboratory identification of fungal species. Some fungal species, primarily macrofungi were identified directly in field based on description Karadžić (2010) and Hagara (2014).

Figure 1. A – Investigated localities in Montenegro (*Google Earth*), B – Isolated fungi on MEA media, ready for identification and preservation on agar slants

Laboratory identification of species was performed based on preparing temporary histological sections for microfungi or isolation and identification of pure cultures for macrofungi (Figure 1). Preparation of histological sections, isolation and identification of fungi was according Muntanola Cvetković (1990). Malt extract agar media (MEA, LAB M UK) was used for the isolation of fungi (Figure 1). Media was prepared according to manufacturer's protocol.

Identification of macrofungi through the characteristics of pure cultures was performed according to descriptions Nobles (1948; 1965) and Stalpers (1978). Identification of microfungi was performed through the characteristics of

microstructures based on descriptions Dennis (1978); Sutton (1980) and Ellis and Ellis (1985).

RESULTS AND DISCUSSION

Investigation revealed 21 fungal species on wych elm (*Ulmus glabra*) in Montenegro (Table 1).

Table 1. Fungi found on wych elm in Montenegro

Fungal species	Type of disease	Colonized part of tree
***Armillaria mellea* (Vahl. Ex Fr.) P. Kummer**	Root rot	Root
***Aurantiporus fissilis* (Berk. & M.A. Curtis) H. Jahn ex Ryvarden**	Heart rot	Trunk
***Bjerkandera adusta* (Willd.) P. Karst.**	Sap rot	Trunk
***Daldinia concentrica* (Bolton) Ces. & De Not.**	Sap rot	Trunk
***Hypoxylon multiforme* (Fr.) Fr.**	Sap rot	Trunk, Branches
***Fomes fomentarius* (L.) Fr.**	Heart rot	Trunk
***Fomitopsis pinicola* (Sw.) P. Karst.**	Heart rot	Trunk
***Ganoderma applanatum* (Pers.) Pat.**	Heart rot	Trunk
***Hypoxylon rubiginosum* (Pers.) Fr.**	Sap rot	Trunk
***Irpex lacteus* (Fr.) Fr.**	Sap rot	Trunk
***Ischnoderma resinosum* (Schrad.) P. Karst.**	Sap rot	Trunk
***Ophiostoma novo-ulmi* Brasier**	Wilt	Trunk, Branches, Root
***Phylloporia ribis* (Schumach.) Ryvarden**	Heart rot	Trunk
***Polyporus squamosus* (Huds.) Fr.**	Heart rot	Trunk
***Mycosphaerella ulmi* Klebahn**	Leaf spot	Leaves
***Pleurotus cornucopie* (Paulet) Rolland**	-	Trunk, Fallen branches
***Stegophora ulmea* (Schwein.) P. Syd. & Syd.**	Leaf spot	Leaves
***Stereum hirsutum* (Willd.) Pers.**	Sap rot	Trunk, Fallen branches
Trametes gibbosa (Pers.) Fr.	Heart rot	Trunk
***Xylaria polymorpha* (Pers.) Grev.**	Sap rot	Trunk
***Schizophyllum commune* Fr.**	Sap rot	Trunk, Stumps

On wych elm (*Ulmus glabra*) trees, 21 fungal species was found, where by 1 fungal species was found on rot, 2 fungal species were found on leaves, 18 species were found on trunk and 3 species were found simultaneously on trunk and fallen branches (Table 1). The most common species were *Armillaria mellea*, *Daldinia concentrica*, *Fomes fomentarius*, *Ganoderma applanatum*, *Polyporus squamosus*, *Mycosphaerella ulmi*, *Ophiostoma novo-ulmi*, *Pleurotus cornucopie* and *Stegophora ulmea*. Species *Ophiostoma novo-ulmi* was the most common, distributed over entire areal of wych elm (*Ulmus glabra*) in Montenegro. The most important species are *Ophiostoma novo-ulmi* and heart rot fungi. Besides *Ophiostoma novo-ulmi* sa the most important pathogen, heart rot fungi were major pathogens on older trees that avoided Dutch elm disease (Table 1).

Some representative examples of found fungal species on wych elm's (*Ulmus glabra*) trunk in Montenegro are showed in Figure 2, Figure 3, Figure 4 and Figure 5.

Figure 2. Some fungi found on wych elm: A – *Armillaria mellea*; B – *Daldinia concentrica*; C – *Bjerkandera adusta*

Figure 3. Some fungi found on wych elm: A – *Hypoxylon multiforme*; B – *Fomes fomentarius*; C – *Fomitopsis pinicola*

Figure 4. Some fungi found on wych elm: A – *Ganoderma applanatum*; B – *Hypoxylon rubiginosum* and *Irpex lacteus*; C – *Ischnoderma resinosum*; D – *Phylloporia ribis*; E – *Polyporus squamosus*; F – *Trametes gibbosa*

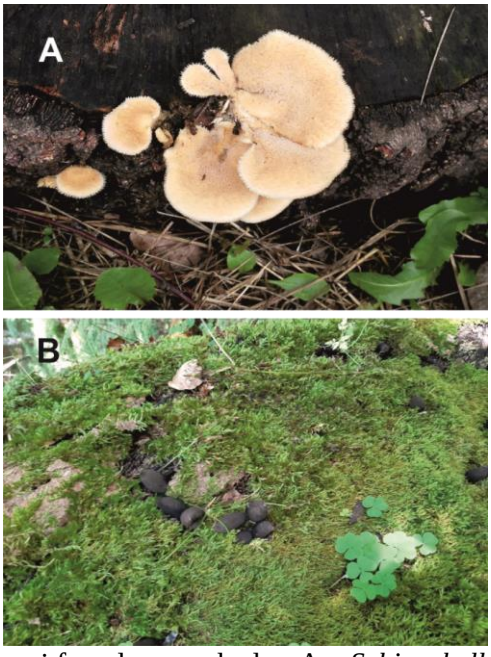

Figure 5. Some fungi found on wych elm: A – *Schizophyllum commune*; B – *Xylaria polymorpha*

On the other side, fungi occurring on leaves causing spots or occurring on entire habitus of trees are showed in Figure 6. All species were found on the trees of different categories of development.

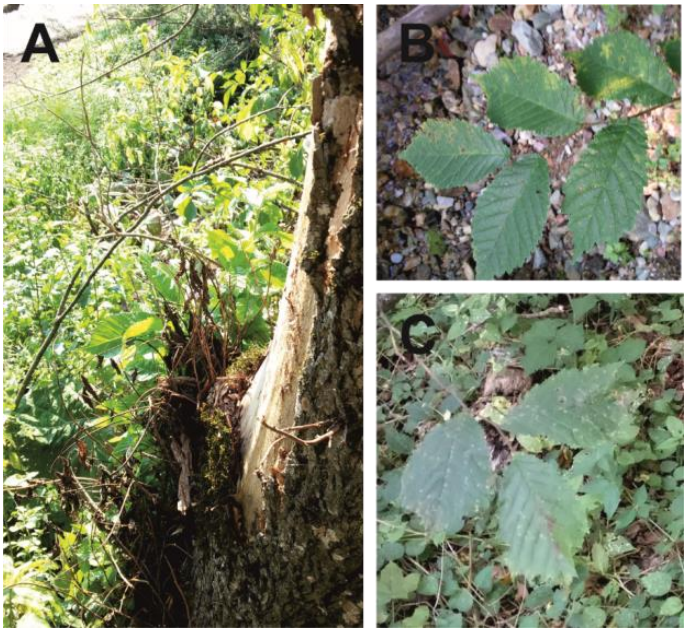

Figure 6. Some fungi found on wych elm: A – *Ophiostoma novo-ulmi*; B – *Mycosphaerella ulmi*; *Stegophora ulmea*

From the investigated fungal species, 12 species were found for the first time on wych elm in Montenegro (Table 2).

Table 2. Fungi found for the first time on wych elm in Montenegro

Fungal species	Type of disease	Colonized part of tree
***Aurantiporus fissilis* (Berk. & M.A. Curtis) H. Jahn ex Ryvarden**	Heart rot	Trunk
***Bjerkandera adusta* (Willd.) P. Karst.**	Sap rot	Trunk
***Daldinia concentrica* (Bolton) Ces. & De Not.**	Sap rot	Trunk
***Hypoxylon multiforme* (Fr.) Fr.**	Sap rot	Trunk, Branches
***Hypoxylon rubiginosum* (Pers.) Fr.**	Sap rot	Trunk
***Irpex lacteus* (Fr.) Fr.**	Sap rot	Trunk
***Ischnoderma resinosum* (Schrad.) P. Karst.**	Sap rot	Trunk
***Phylloporia ribis* (Schumach.) Ryvarden**	Heart rot	Trunk
***Mycosphaerella ulmi* Klebahn**	Leaf spot	Leaves
***Stegophora ulmea* (Schwein.) P. Syd. & Syd.**	Leaf spot	Leaves
Trametes gibbosa (Pers.) Fr.	Heart rot	Trunk
***Xylaria polymorpha* (Pers.) Grev.**	Sap rot	Trunk

Recorded fungi on wych elm (*Ulmus glabra*) revealed high presence of different species. This way, the first null hypothesis was rejected and alternative hypothesis that majority of recorded species are for the first time found on wych elm (*Ulmus glabra*) trees in Montenegro was accepted. Mycological complex of this tree species was significantly revealed in this part of wych elm (*Ulmus glabra*) areal.

Sap rot and heart rot fungi were detected. Sap rot and heart rot fungi have different survival strategies, sap rot fungi use "ruderal strategy", primarily are focused on fresh, injured substrate (Vasaitis, 2013). Rot characterized by investigated fungi was easily recognized through presence of their fruit bodies in advanced stages of decay (Figures 2-5). From Table 1 it can be seen that majority of heart rot fungi on wych elm (*Ulmus glabra*) follow Haddow-Etheridge concept (Vasaitis, 2013). This concept represents type of fungal heart rot development that is triggered by naturally aging of trees or other natural factors influencing tree vitality. Fungi *Fomes fomentarius*, *Fomitopsis pinocola*, *Polyporus squamosus* and *Ganoderma applanatum* colonize wide range of broadleaved hosts (Karadžić *et al*., 2016; Vemić and Milenković, 2018; Radulović *et al*., 2020; Karadžić *et al*., 2020). Trees that are in early stages or avoided Dutche elm disease had fungal infection associated either with their injury or aging based on these results. Heart rot fungi associated with Haddow-Etheridge concept are mostly host specified (Vasaitis, 2013). However, majority of found heart rot fungi, primarily species *Ganoderma applanatum*, *Fomes fomentarius*, *Fomitopsis pinocola* and *Polyporus squamosus* can be found on wide ranges of trees hosts (Hagara, 2014). This makes protection strategies difficult but more concrete in the same time due to knowledge about identified fungal species.

Also, symptoms on leaves were specific for fungi *Mycosphaerella ulmi* and *Stegophora ulmea* (Figure 6 B, C) and also served for identification in addition to their morphological characteristics. Symptoms of *Mycosphaerella ulmi* were typical red and brown spots on leaves, often mixed with chlorosis and discoloration of leaves (Figure 6 B). Symptoms of *Stegophora ulmea* were typical black tar spots, pale at the beginning and tyrning black with mature (Figure 6 C). Spots were typical for this species and identification is often possible just based on symptoms. In scientific literature it is unclear if this species is synonym for *Systrema ulmi* (Schleicher). In this research we consider this to be the same species based on symptoms occurring and morphological characteristics of fungi.

Symptoms from *Ophiostoma novo-ulmi* were characteristics for this species (Figure 6 A). One of the first symptoms was wilting of thin branches, which soon affected entire tree (Figure 6 A). Formal opinion is that wych elm (*Ulmus glabra*) is more infested by Dutch elm disease than the field elm based on early researches (Townsend, 1971; Brasier, 1977) as well as recent research about clonal resistance (Solla *et al*., 2005). However, experiences from foreign researches also showed that in some cases the field elm (*Ulmus minor*) trees can be more endangered by epidemic (Łakomy *et al*., 2016). During this research heavy wilting was recorded on both wych elm (*Ulmus glabra*) and the field elm

(*Ulmus minor* Mill.). In this point it was hard to evaluate which species declines faster in these ecological conditions because it is also impacted by different subtypes of *Ophiostoma novo-ulmi* present in this part of areal.

Recorded fungal species in this study were found on trees damaged due to abiotic factors, wilting caused by *Ophiostoma novo-ulmi* and decay caused by *Ganoderma applanatum*, *Fomes fomentarius*, *Fomitopsis pinocola* and *Polyporus squamosus*. Interestingly, some typical secondary colonizers *Auricularia auricula-judae* (Bull. ex St-Amans) Wettst and *Tremella mesenterica* Retz. ex Hook were not found in this investigation. These species are often found on declining broadleaf trees species in domestic forests (Radulović *et al*., 2022). These results rejected the second null hypotheses and accepted alternative hypothesis that fungi occur in succession on infected wych elm (*Ulmus glabra*) trees in Montenegro. Results revealed that trunk is the most endangered part of trees with majority of found fungi (Table 1, Table 2). These findings rejected the third null hypothesis that there isn't difference in distribution of fungal damages on different organs of wych elm (*Ulmus glabra*) trees in Montenegro. Alternative hypothesis that there is difference in distribution of fungal damages on different organs of wych elm (*Ulmus glabra*) trees in Montenegro was accepted.

Highly pathogenic species *Botryodiplodia hypodermia* was not found in this investigation. This fungus causes elm dieback and is potential factor endangering elms in Europe (Bartnik *et al*., 2022). Possible explanation is because *Botryodiplodia hypodermia* is much less prevalent in Europe than in North America (Bartnik *et al*., 2018). Also, forest stands and climate characteristics in Montenegro are somewhat different than in other geographically distant parts of Europe. However, it is possible that species was present and unsuccessful isolations were due to decrease of mycelium activity in tissues. Also, cancer symptoms that remind on this species were not detected during the investigation.

Montenegro is one of the rarest countries that have fully grown wych elm (*Ulmus glabra*) trees, left to spontaneous development without Dutch elm disease, enabling researches about other fungi associated with trees decline. Production of trees material that is more tolerant to Dutch elm disease and also doesn't have special susceptibility to other pathogens is more possible based on these results. Practically, on global scale, production of trees material that is more tolerant to mechanical damages that favorize development of sap rot fungi *Bjerkandera adusta*, *Daldinia concentrica*, *Hypoxylon multiforme*, *Hypoxylon rubiginosum*, *Irpex lacteus*, *Ischnoderma resinosum*, *Stereum hirsutum*, *Xylaria polymorpha* and *Schizophyllum commune* should be done. The usage of proveniences with thicker bark and more dense wood should be more intensive for breeding programs. In modern forestry genetic engineering is important factor in disease management (Edmonds, 2013). Also, wych elm (*Ulmus glabra*) as the other admixed species in beech forests requires different strategies and programs in order to achieve diversity of these forests (Schulze *et al*., 2016).

In domestic forests, integral protection is the most applicable protection method. Between everything, this means measures against Dutch elm disease should also be focused on other fungi. Because majority of found species are less host specified this means that protection strategies should be focused on other trees species occurring with wych elm (*Ulmus glabra*), primarily beech (*Fagus sylvatica* L.). Operations like thinning and pruning should be more intense in domestic beech forests to avoid transmission of inoculum. Also, the other forest operations should be performed more precisely to avoid damaging of trees and making entry points for sap rot fungi.

CONCLUSIONS

Prior to this research there were no detailed studies about fungal pathogens on wych elm (*Ulmus glabra*) in Montenegro. Based on performed research all conclusions can be pointed on next way:

On wych elm (*Ulmus glabra*) there were found 21 fungal species. Based on part of tree, 1 species was found on rot and base of tree, 2 species were found on leaves, 18 species were found on trunk and 3 species were found simultaneously on trunk, branches or root.

Majority of species occurs in succession. Often necrotic spots on leaves caused by *Mycosphaerella ulmi* and *Stegophora ulmi* (*Systrema ulmi*) leads to physiological stress in trees allowing *Armillaria mellea* and other rot fungi to develop active infections and cause decline of trees that avoided infection of *Ophiostoma novo-ulmi*.

Species *Mycosphaerella ulmi* and *Stegophora ulmi* (*Systrema ulmi*) were also found solitarily on leaves on youngest seedlings. Mortality of young wych elm (*Ulmus glabra*) specimens were recorded in some cases. In other cases, young wych elm (*Ulmus glabra*) continued development but it is still unclear about further health condition on this these trees.

Sap and heart rot fungi were the most diverse group of fungi colonizing wych elm (*Ulmus glabra*). Trees that had some form of resistance or "disease escape" from Dutch elm disease were severe damaged by these pathogens.

Species *Ophiostoma novo-ulmi* was still the most important pathogen of wych elm (*Ulmus glabra*) causing the wilting of trees across entire country.

Majority of fungal species, 12 species were found for the first time on wych elm (*Ulmus glabra*) in Montenegro. These results will contribute to knowledge about mycoflora of wych elm (*Ulmus glabra*) in Montenegro.

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IDENTIFICATION OF SELF INCOMPATIBILITY GENOTYPES IN SWEET CHERRY COMMERCIAL CULTIVARS

SUMMARY

Sweet cherry exhibits RNase based gametophytic self-incompatibility system which is controlled by a single multi-allelic locus S. The identification of the self-(in) compatibility genotypes facilitates orchard management and is important for future breeding programmes. However, to ensure an effective pollination and fruit set compatible cultivars should have overlapping flowering time. The PCR-based detection of S-alleles was carried out in seventeen commercial sweet cherry cultivars of foreign origin by using two consensus primers for the RNase gene and two specific primers for the confirmation of the S_3 and S_4 allele. Totally, eight different S-alleles in ten S locus combinations were detected. 35.3 % of cultivars were self-compatible, whereas 59 % were assigned into seven individual self-incompatibility groups. S-alleles distribution linked with flowering and fruit ripening time was also analyzed. Most S-alleles (7) were identified in the mid-early blooming cultivars where the most frequent were S_3; S_4 and S_1.

On the other hand, late blooming cherry cultivars had the S_3S_{12} genotype, where the S_{12} allele was specific to these cultivars. The most frequent allele in the early fruit ripening cultivars was the allele S_4. The S_{14} allele was specific of cultivars with medium fruit ripening time while the alleles S_6 and S_{12} were detected only in the late fruit ripening cultivars. The synchronization of harvesting time within a commercial orchard would benefit to growers. Thus, identifying S genotype compatible cultivars, with the overlapping flowering and fruit ripening time would enable better orchard management.

Keywords: S-alleles, Sweet cherry, Self-incompatibility, flowering time, Albania

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INTRODUCTION

Sweet cherry (*Prunus avium* L.) is an economically important fruit species cultivated for its edible fruit used mainly for fresh consumption, jam, liqueur production and as ingredient in different processed foods. The cultivar structure in Albanian orchards had changed over years, affected by the consummator and trade preferences, leading to the introduction of several foreign cultivars. Successful pollination of the flowers is a requirement for a high yield in sweet cherry production. Many other factors, such as the same blooming time, climate conditions, the presence of pollinators and the right pollen donor are crucial factors for fertilization (Schuster *et al*., 2007).

Prunus avium L. exhibits an RNase gametophytic SI system (GSI), controlled by a single multi-allelic locus called S-locus (Tao *et al.,* 1999). Fertilization can only occur when the S-haplotype expressed by pollen and pistil are different (McCubbin and Kao, 2000). Cultivars showing the same S-genotype belong to the same incompatibility group, they cannot pollinate each other as they are cross-incompatible. In self-incompatible cultivars as sweet cherry, a self-incompatible cultivar must be planed with a pollinator one to ensure fruit set (Ikeda *et al*., 2004) and two to three cross-compatible cultivars must be planted to achieve effective cross-pollination (Schuster *et al.,* 2007). Therefore, the knowledge of the cross-incompatibility groups between sweet cherry cultivars is useful for growers, and breeders especially when specific crosses between two cultivars are desired.

S-allele genotyping employing molecular PCR methods has become a useful tool for the rapid determination of the incompatibility groups in sweet cherry cultivars (Patzak *et al*., 2019a). To identify S-RNase alleles several specific and consensus primers that amplify two S-RNase introns were designed (Tao *et al.,* 1999; Wiersma *et al*., 2001; Sonneveld *et al*., 2003; Sharma *et al*., 2014). In addition, Sonneveld et al. (2006) designed florescent primers that amplify first intron alleles enabling precise identification of alleles and accurate S-incompatibility group assignment. Several studies have addressed S-incompatibility evaluation and S-allele identification in sweet cherry cultivars grown in different countries employing PCR based S allele typing (Sonneveld *et al*., 2003; Ershadi and Moghadam, 2009, Ipek *et al*., 2011, Ganopoulos *et al,*. 2012, Sharma *et al*., 2014, Cachi and Wunsch, 2014). A total of 22 different S-alleles, 63 incompatibility groups and 91 self-compatible sweet cherries have been defined so far and reported in a summarized list of 1483 cultivars by Schuster (2012, updated 2020).

S-allele constitution in commercial sweet cherry cultivars in Albania have not been previously defined. The present study aims the characterization of S-allele combination, the identification of self-incompatibility groups of sweet cherry cultivars that constitute most of the commercial orchards by employing PCR based method. In addition, the distribution of the S-alleles linked with the flowering and fruit ripening time will be investigated to provide valuable insights into the compatible cultivars with overlapping flowering and fruit ripening time.

MATERIAL AND METHODS

Seventeen accessions of sweet cherries, *Prunus avium* L., of foreign origin well adapted and widely grown in local orchards were collected, their list and origin are given in table 1. The genomic DNA was isolated from 100-120mg fresh leaves using CTAB method described by Kump and Javornik (1996). The amplification was carried out using the consensus primers for the first and second intron, PaConsI (Sonneveld *et al*., 2003, 2006) and PaConsII (Sonneveld *et al*., 2003), respectively. The presence of the allele S3 and S4 were confirmed by using the allelic specific primers PasS3 and PaS4 (Sharma *et al*., 2014)) The PCR reaction was performed in a total volume of 15µl containing 1xPCR buffer, 2.5mM $MgCl_2$, 0.2mM dNTPs, 0.2mM each primer, 0.4U Taq polymerase (New England BioLabs). The reaction conditions for 1st intron consensus primer PaConsI were as follow: denaturation 94°C for 2 min, 10 cycles of 94°C for 10 sec, 58°C for 2 min, 68°C for 2 min, followed by 25 cycles of and 72°C for 5 min (Sonveld *et al.*, 2006), while for the 2nd intron consensus primer PaConsII, the amplification was carried out in 94°C for 2 min, 10 cycles of 94°C for 10s and 68°C for 2 min, followed by 25 cycles of 94°C for 10 sec, 58°C for 2 min and the final extension at 68°C for 2 min increasing 10 sec for each cycle (Sonneveld *et al.*, 2003). The thermal conditions for specific primers PaS4 and PaS3 were initial denaturation 95°C for 2 min, 36 cycles of 30 sec at 94°C, 45 sec at 58-61°C, 2 min at 72°C and final elongation 72°C 10 min (Sharma *et al.*, 2014). The PCR products were run in different concentration of agarose gel in 1x TAE, stained with ethidium bromide at 9 v/cm for 40 to 60 min; 2% agarose gel for the PaConsI-F/PaConsI-R2, 1.3% agarose gel for PaConsII-F/PaConsII-R, while the PCR amplification products of specific primers PaS3 and PaS4were differentiated in 1.2 % agarose gel. The images were captured with GeneSmart UV gel documentation system and the size estimation of fragments was performed against 1kb standard using PyElph version 1.4 software.

The correlation between distribution of the S alleles and ripening time of the cherry fruit was investigated. The ripening period data about fourteen cherry cultivars used in this study was previously studied (Lazaj and Ferraj, 2020) and included a time span of five weeks, from April to June.

RESULTS AND DISCUSSION

The analysis of 17 sweet cherry cultivars with S locus consensus and specific primers for RNase first and second intron, identified in total eight different S-alleles in ten locus combinations (Table 1).

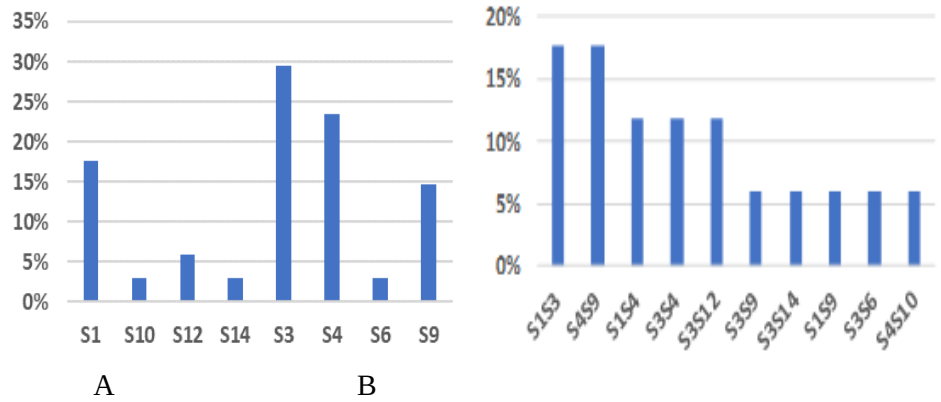
The size of S alleles generated by the amplification of our sample set with first intron consensus PaConsI primer ranged from 230-450 bp. The low difference between fragments some alleles had similar size, hence their accurate identification was difficult. However, the PCR products obtained using the second intron consensus PaConsII primer ranged from 570 - 2300bp, herein the S-allele identification was easier compared with the first intron amplified fragments, since the fragments run distantly from each other in the agarose gel

enabling accurate discrimination. Furthermore, the specific PaS3 and PaS4 primers were used to confirm the presence of these two specific alleles, the estimated size of obtained S3 and S4 alleles was 325bp, and 390bp, respectively. The allele had the expected sizes as reported by Sharma *et al.* (2014) confirming their presence in studied cherries genotypes.

Table 1. S genotypes and self-in compatibility groups of sweet cherry cultivars

Cultivar	Origin	S locus genotype determined with consensus primers	S3 and S4 specific primers confirmation		(In)compatibility group
			S_3	S_4	
Celeste	Canada	$S_1S_{4'}$		+	SC
Grace Star	Italy	$S_{4'}S_9$		+	SC
Mora di Cazzano	Italy	S_3S_{14}	+		LIV
Ferrovia	Italy	S_3S_{12}	+		XXII
Lala Star	Italy	S_1S_3	+		II
Regina	Germany	S_1S_3	+		II
Lapins	Canada	$S_1S_{4'}$		+	SC
Black Star	Italy	S_1S_3			II
New Star	Canada	$S_3S_{4'}$	+	+	SC
Burlat	France	S_3S_9	+		XVI
Kordia	Czech Republic	S_3S_6	+		VI
Feu 5	France	S_4S_{10}		+	-
Sweet Heart	Canada	S_3S_4	+		SC
Schneider	Germany	S_3S_{12}	+		XXII
Sweet Early	Italy	S_1S_9			XVIII
Big Star	Spain	$S_{4'}S_9$		+	SC
Crazy Star	Italy	S_4S_9		+	XXI

The analysis identified a total of eight different alleles, the most frequent were S_3 (29.4%), S_4 (23.4%), S_1 (17.6%) and the allele S_9 (14.7%), followed by S_{12} (5.9%). The rarest alleles were the alleles S_6, S_{10}, $S_{14,}$ which were identified only once in three different cultivars 'Kordia', 'Feu 5' and 'Mora di Cazzano', respectively (Figure 1). The number of detected S alleles is considered high considering the small number of genotypes analyzed (17 sweet cherry genotypes). However, high diversity of S alleles observed in present study might be because of the different origin of the analyzed cherry genotypes.



A B

Figure 1. Frequency of S alleles is sweet cherry cultivars (A), and S genotypes in sweet cherry cultivars (B)

Across 17 sweet cherry cultivars there were identified ten allele combinations (Table 1, Figure 1). Genotypes S_1S_3 and S_4S_9 had a frequency of 17.6%, followed by S_1S_4, S_3S_4 and S_3S_{12} with a frequency of 12%. The genotypes S_3S_9, S_3S_{14}, S_1S_9, S_3S_6 and S_4S_{10} were detected only in once in 'Burlat', 'Mora di Cazzano', 'Sweet Early', 'Kordia', 'Feu 5', respectively. A total of eight groups of incompatibility were found from 47 groups reported by Schuster M (2012). Six cultivars were classified as self-compatible (SC), 'Celeste', 'Grace Star', 'Lapins', 'New Star', 'Sweet Heart' and 'Big Star', ten cultivars resulted incompatible, while Feu5 was not assigned to any group presented by Schuster M. (2012, updated 2020) (Table 1).

The most frequent incompatibility groups were II (S_1S_3) and XXII (S_3S_{12}) with 18.8% and 12.5% percent, respectively. Whereas the other groups of incompatibility groups had a frequency of 6.3% each.

To obtain a successful pollination the S-alleles constitution of sweet cherry cultivars within production orchards is crucial, however the overlapping of blooming periods of the cultivars within the orchards is also relevant to obtain a high rate of pollination (Patzak *et al*., 2019b).

Therefore, the data obtained in a previous study on the time of flowering and fruit ripening time (Lazaj and Ferraj, 2020) on fourteen cultivars included in our study were used as a basis to investigate the distribution of S alleles linked to blooming and fruit ripening time.

The classification of 14 out of our 17 sample set of sweet cherry cultivars based on the data published by Lazaj and Ferraj (2020), according to the flowering onset and ripening time was given in the table 2.

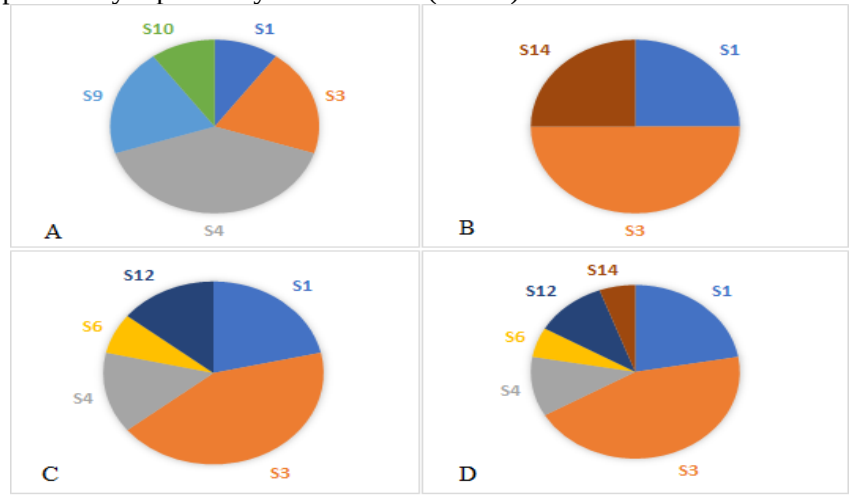
The correlation between distribution of the S alleles and ripening time of the cherry fruit was investigated. The ripening period data about the cherry cultivars was previously studied (Lazaj and Ferraj, 2020) and included a time span of five weeks, from from April 28th to June 6th.

Table 2. Ripening period of cultivars

Cultivars		
Early ripening	**Medium ripening**	**Late ripening**
Burlat	Black Star	Lala Star
Feu 5	Mora di Cazzano	Lapins
Celeste		Kordia
Grace Star		Ferrovia
New Star		Sweet Heart
		Regina
		Schneiders
Cultivars		
Early flowering	**Mid flowering**	**Late flowering**
Burlat	Lapins	Schneiders
Black Star	Mora di Cazzano	Ferrovia
Feu 5	Celeste	
Grace Star	New Star	
Regina	Kordia	
Sweet Heart		
Lala Star		

**data on fruit ripening and flowering time of sweet cherry culticars were obtained by Lazaj and Ferraj (2020)*

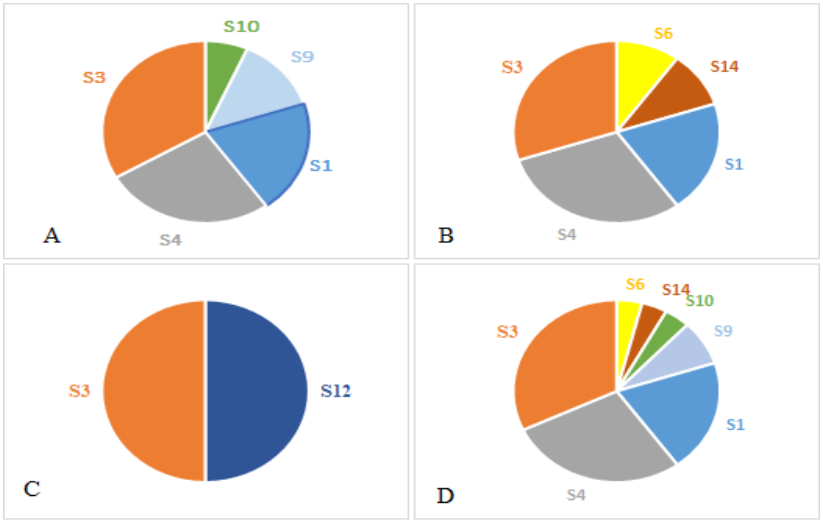
The most frequent allele in the early season ripening cultivars was S_4 while the allele S_9 and S_{10} were detected only in cultivars with early ripening in the 1st, 2nd and 3rd week. The allele S_{14} was detected only in cultivars with medium ripening period in the 3rd to 4th week. The alleles S6 and S12 were detected only is late ripening cultivars from the 3rd to 5th week (Figure 2). The occurrence of S_9 in the 2nd and 3rd week of cherry ripening and of the S_{12} allele in the 3rd to 6th was also previously reported by Patzak *et al*. (2019b).



**A-early; B-medium; C-late; D-medium-late fruit ripening*

Figure 2. S-alleles distribution of cherry cultivars linked with ripening time

The most S-alleles (7) were found in the mid-early blooming cultivars in which the flowering time overlap. Overlapping in flowering period is a critical element in the cultivar composition of cherry orchards as these cultivars are considered suitable for crosses if their S-genotype is compatible (Radičević *et. al*., 2015). In these cultivars the most frequent S-alleles were S_3; S_4 and S_1 (Figure 3).



**A-early; B-medium; C-late; D-early-mid flowering time*

Figure 3. S-alleles distribution of cherry cultivars linked with flowering time

The allele S_{10} was detected only in early blooming cultivars (‘Feu 5’) while S_{14} and S_6 allele were detected only in mid-flowering period cultivars ‘Mora di Cazzano’ and ‘Kordia’, respectively. On the other hand, late blooming cherry cultivars had S_3S_{12} genotype and the S12 allele was found only in these cultivars (‘Shneiders’ and ‘Ferrovia’).

CONCLUSIONS

Sweet cherry is an economically important fruit tree species, widely cultivated for its edible fruits. The high rate of successful pollination would provide greater production of fruits and benefits for growers. The harmonization of the S-compatibility cultivars with the flowering time would provide better management of the orchards. Therefore, 17 sweet cherry cultivars were analyzed by S locus consensus and specific primers for the S-RNase gene to determine their composition of the S-allele and identifying self-incompatibility genotypes. Herein, we report the identification of ten S-allele and seven groups of self-incompatibility identified across 17 analyzed cultivars. The distribution of the s-alleles linked with the flowering and fruit ripening time for 14 of the analyzed cultivars has provided valuable insights into the compatible cultivars with overlapping flowering and fruit ripening time. This study represents the first S-genotype characterization of sweet cherry cultivars in Albania which will be of

use as reference for future wider studies in S-allele detection as well as in future selection of individuals for cross -fertilization in breeding programmes.

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STRUCTURE COMPONENTS AND YIELDING CAPACITY OF *CAMELINA SATIVA* IN UKRAINE

SUMMARY

The results of three–year research on the influence of different fertilization levels and foliar fertilization with micronutrients and growth regulators on the formation of structure elements and yielding capacity of *Camelina sativa* variety Hirsky are presented, their parameters in the process of plant vegetation has been defined. The influence of mineral fertilizers and micro–fertilizers on the elements composing the structure and formation of *Camelina sativa* seed yield on low–fertile sod–podzolic soils of Precarpathians of Ukraine has been studied. It was found that application of mineral fertilizers increases yielding capacity of *Camelina sativa* by 56–87%, and together with three additional fertilizations of plants during vegetation period – almost by 4 times (up to 1.99 t.ha^{-1}, which is 0.96 t ha^{-1} more than in the control).

Optimal parameters of cultivation technology elements of *Camelina sativa*, which allow to obtain seed yielding capacity at the level of 1.78–2.04 t.ha^{-1}, have been determined. On the basis of analysis of relation between yielding capacity and individual indices of *Camelina sativa* structure it was determined that the closest relation was between yielding capacity and the number of pods (R = 0.967). Therefore, it is the number of pods that allow the most accurate prediction

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of yielding capacity level for *Camelina sativa* a according to calculated regression equation.

Keywords: *Camelina sativa*, yield structure, seed yielding capacity, mineral fertilizers, micro–fertilizers

INTRODUCTION

Camelina sativa is a long–forgotten, uncommon, promising spring and winter oil crop of the *Brassicaceae* family of the genus *Camelina*. No wonder it is one of the oldest cultures, originating from Western Europe (Megaloudi and Fragkiska 2006; Hryhoriv *et al.*, 2020). Today, red and winter red are grown in almost all countries of the world, but each of them has its own leading oilseed crop. Today, Austria, Great Britain, Denmark, Germany, Russia, USA, Finland and France conduct active works on selection and agro–technics of *Camelina sativa* as oil plant, and the sown area under it is constantly growing (Madhav *et al.*, 2020; Landré *et al.*, 2020; Yakupoglu *et al.*, 2021; Hryhoriv *et al.*, 2021a).

Today, ryegrass is grown in Ukraine on small areas in the Polissya and Forest–Steppe zones – about 5–6 thousand hectares, which is only 3% of all oilseeds, but this crop has significant prospects for development as a highly productive oilseed crop. Although looking at the prospects of culture, it is safe to say that in the near future these areas may be increased by 3–4 times. Today, the leaders in growing ryegrass in Ukraine are Sumy, Chernihiv, Kyiv and Cherkasy regions, although there are all the prerequisites for expanding the area under its crops throughout Ukraine (Hamayunova, 2017; Long *et al.*, 2018; Hryhoriv *et al.*, 2021b; Tonkha *et al.*, 2021; Rieznik *et al*,. 2021). Recently, *Camelina sativa* has become the subject of various experiments aimed at assessing its future potential.

The culture is promising for growing in arid conditions of the steppe zone of our country, as it is undemanding to growing conditions, cold- and drought-resistant, has a unique resistance to oilseeds to disease and pests. It should be noted that the interest in *Camelina sativa* is explained by the fact that it successfully combines high potential of seed yielding capacity (2.0 $t.ha^{-1}$ in Canada, 2.1–2.2 $t.ha^{-1}$ in Ireland in 2013–2015) and unique properties and composition of *Camelina sativa* oil: healthy composition of fatty acids, high content of vitamins, high resistance to oxidation (Prianyshnykov, 1963; Volokh *at al.*, 2005; Hryhoriv *at al.*, 2020).

Currently, a very promising new direction of *Camelina sativa* use is being developed – to obtain environmentally friendly renewable fuel, biodiesel. *Camelina sativa* is promising for processing into biodiesel thanks to relatively high content of long–chain fatty acids (eicosenoic and erucic, in total up to 17–26%), which are characterized by high heat of

combustion. Today, the world leader in rice cultivation is Russia, where over the past 7 years the area under it has increased from 12 thousand hectares to 540000 hectares, of which 463000 tons of rice oil per year. Most of the oil produced is a raw material for the production of aviation biodiesel. Finland is the main producer of biodiesel from Russian raw materials (aviation biokerosene), the buyer of which is Lauftganza (Putnam, 1993; Frohlich, 2005; Hamayunova, 2017; Lü *et al.*, 2019; Hryhoriv *et al.*, 2021c).

Not for nothing, *Camelina sativa* has many parameters which determine its commercial attractiveness as oil and industrial crop. First, it is a precocious crop that allow to increase seasonal load on combine harvesters, and its early harvesting creates conditions for struggle with littering of fields in the long post–harvest period and allows to prepare the soil for the next winter and spring crops. Secondly, cultivation of *Camelina sativa* is relatively low cost.

Resistance of *Camelina sativa* to diseases and pests can reduce the cost of insecticides by 2–3 times compared to other crops of the cabbage family (*Brassica napus*, *Barbaréa vulgáris*), which is a significant item of savings at current prices (Komarova, 2003; Hryhoriv *et al.*, 2021a).

MATERIAL AND METHODS

Field research was conducted on the research base of the Department of Technology of Growing, Seed Production and Biochemistry of Cruciferous Crops of the Precarpathian State Agricultural Research Station of the Institute of Agriculture of the Carpathian Region NAASU on sod podzolic soil during 2018–2021.

The soils of experimental plot are turf deep podzolic gleyed, by mechanical composition – coarse-grained heavy–clayed with strong humus horizon up to 75 cm and are characterized by the following indices: acidity, pH – 5.2, humus content (%) – 2.74, soil content of main nutrient elements ($mg.kg^{-1}$): nitrogen – 85, phosphorus – 47.0, potassium – 117.0.

Precursor – winter wheat. The sowing was carried out according to experimental scheme. For sowing was used a variety Hirsky, selection of institute AIP. Because scientists have found that *Camelina sativa* is not sensitive to potassium fertilizers (Poliakov, 2011; Hryhoriv *at al.*, 2020), so we studied the effects of only nitrogen and phosphorus fertilizers. In the experiment, mineral fertilizers in the form of nitrogen phosphorus and potassium, ammonium nitrate and granular superphosphate were applied to main soil tillage according to the following scheme:

Control – without fertilizers;
Background – ($N_0P_{45}K_{45}$);
Background – ($N_{30}P_{45}K_{45}$);
Background – ($N_{30}P_{45}K_{45}$) + N_{60};

Background – ($N_{30}P_{45}K_{45}$) + Vympel (500 g.ha^{-1}) + Oracul multicomplex (1 l.ha^{-1}) + Oracul colamine boron (1 l.ha^{-1}) + Oracul sulfur active (2 l.ha^{-1}).

The experiment was based on four repetitions; the area of accounting plot – 20 m^2. A variant without fertilizers was the control.

Fertilization of *Camelina sativa* crops was carried out with nitrogen fertilizers, micro–fertilizers and growth stimulants according to corresponding variants of experimental scheme in the rosette phase.

In the experiment was sown *Camelina sativa* variety Hirsky of selection Ivano–Frankivsk Institute AIP NAAS included in the State Register of varieties suitable for cultivation in Ukraine and adapted to the conditions of the Carpathians. The seed yield stated in the patent is on average 2.1 t.ha^{-1}, green mass – 40.5 t.ha^{-1} (Abramyk *et al.*, 2003; Karpenko *et al.*, 2019).

Agrotechnics of *Camelina sativa* cultivation on research sites was generally accepted for soil and climatic conditions of Prykarpattia, except for the studied factors (Syvyryn & Reshetnykov, 1988).

Weather conditions in Prykarpattia are formed under the influence of three main factors of geographical origin, air circulation and litter surface. An important climatic factor in this region is the Carpathians, which affect the spread of air currents near the earth's surface. Prykarpattia is a moderately warm and humid area. Natural and climatic conditions that have developed in Ivano–Frankivsk region contribute to development of agriculture and forestry, cultivation of main crops.

It should be noted that during the growing season of *Camelina sativa* during the study period, weather conditions in some months differed significantly from the average long-term data both in terms of temperature and rainfall.

However, analyzing the weather conditions, we see that the formation of the yield of *Camelina sativa* occurs with the appropriate satisfaction of plant needs, namely the optimal values of environmental factors, a significant proportion of which are meteorological values.

Thus, we found that during the 2018–2021 study, weather conditions differed slightly from the long-term average in terms of rainfall and temperature, but in some months we observed significant differences, which ultimately affected the level of crop productivity.

In the process of performing the work we used the following research methods: field – to study the interaction of the research subject with biotic and abiotic factors; laboratory – analysis of plants and soil in order to study the interaction between the plant and environmental conditions; measuring and weighing – to establish the level of yield of spring rye; mathematical and statistical – to establish the reliability of the results.

Mathematical processing of obtained analytical digital material was performed by the method of disperse and correlation analysis according to Dospekhov (1985) and Ushkarenko (2014) using the computer program Agrostat 2013.

RESULTS AND DISCUSSION

The structure of the crop is the ratio between main elements of yielding capacity: seeds and straw, aboveground part and root system, etc. And the level of crop yield depends on the number of plants per unit area, pods per plant, seeds and seed weight.

Taking into account the fact that individual elements of the structure are formed at different stages of plant ontogenesis, certain agrotechnical conditions are necessary for their successful development. From the level of providing plants with nitrogen in the phases of rosette formation, stemming, branching and its concentration in vegetative organs, the conditions for seed formation are improved. Fertilizers have a significant effect on the weight of 1000 seeds.

Important indicators that reflect the productivity of *Camelina sativa* are the number of branches, pods per plant, the number of seeds per pod per plant and the weight of 1000 seeds. It is with the help of these indicators you can determine the level of biological yield of the crop. In order to substantiate yield indices obtained under conditions created by the experimental variants, we analyzed yield structure of *Camelina sativa*.

It is known that the highest yield is formed at the optimal ratio of all structural elements. At the same time, with poor development of at least one of the structural elements of productivity, it can be partially compensated by other indicators. However, the productivity of such crops may not always be maximum. Therefore, agricultural techniques for growing crops should ensure uniform and optimal development of all elements of productivity, as only under this condition it is possible to obtain maximum results (Lykhochvor & Petrychenko, 2010).

Table 1. Influence of nutrient elements of camelina glabrata plants on the elements of camelina glabrata crop structure (average for 2018–2021)

Variant	Number of branches, pcs	Number of pods, pcs	Number of seeds per plant, pcs	Weight of 1000 seeds, g
Without fertilizers (control)	5	151	1661	1.01
$P_{45}K_{45}$	7	264	2485	1.06
$N_{30}P_{45}K_{45}$	10	384	3184	1.16
$N_{30}P_{45}K_{45}$+ N_{60}	14	418	3915	1.23
$N_{30}P_{45}K_{45}$+ micro–fertilizers	15	399	3904	1.19
$\bar{X} \pm S_{\bar{x}}$	0.2±3.8	33.1±5.2	0.2±4.5	0.02±7.2

The presented data show that application of mineral fertilizers had positive effect on the crop structure, one of which is the number of pods per plant. Thus, with increasing fertilizer dose, there was an increase in the number of pods per plant, as well as other indices. The studied doses of fertilizers in the experimental variants provided increase in the number of branches per plant from 5 to 15 pcs, the number of pods per plant from 151 to 418 pcs, the number of seeds per plant from 1661 to 3915 pcs, compared to the control without fertilizers. It was established that the number of seeds from a plant changes to the greatest extent

from the structural elements of the formation of individual seed productivity, which was 2485-3915 pieces when fertilizers were applied. That is, the increase in control was 51-152%. Also, it should be noted that the application of nitrogen fertilizers leads to an increase in the number of pods on one plant, but no significant effect on the weight of 1000 seeds was noted. The increased number of seeds per 1 m^2 was caused by an increase in the number of pods per plant, but not by the number of seeds per pod. The level of yield of *Camelina sativa* seeds is directly proportional to the individual productivity of plants, which is determined primarily by the number of pods per plant, pod weight, seed weight per plant and weight of 1000 seeds. We have established that improving the conditions of mineral nutrition with the introduction of fertilizers significantly affects the formation of reproductive organs. Thus, with the introduction of different doses of nitrogen fertilizers in feeding, the number of pods on the plant varied from 418 pcs. – in the variant with application of $N_{30}P_{45}K_{45}+N_{60}$, up to 399 units/plant – in the variant with a dose of $N_{30}P_{45}K_{45}$+microfertilizer, which exceeded the indicator of the variant without fertilizers by 9% and 171%.

The weight of 1000 seeds did not change significantly under the influence of fertilizers 1.01 to 1.23 g.

Our results are also confirmed by the studies of Vakhnenko and Polyakov conducted in the Pidenny Steppe of Ukraine, which indicates that the use of biological preparations contributes to the growth of plant height, the number of pods on one plant, the weight of seeds from one plant and the weight of 1000 pcs. seed (Vakhnenko & Poliakov, 2010).

The results of research indicate that optimization of plant nutrition through the use of rational modern approaches to fertilization has had positive effect on such components of crop structure as the number of branches, the number of pods and seeds per plant and the weight of 1000 seeds. Our observations are confirmed by studies of Hamayunova (2017) conducted in the steppe zone.

A change in indicators of productivity elements led to a change in the yield level. A positive effect of mineral fertilizers and growth stimulants on this indicator was noted for all years of research. The effectiveness of their application was noted on all variants that gave an increase in productivity.

Thanks to optimization of growing conditions by appropriate combination of structural elements of technology, it is possible to achieve maximum realization of genetic variety potential in the economic harvest. As you know, yield is a determining indicator of the feasibility of growing crops and depends primarily on the genetic characteristics of the variety, its response, adaptation to soil and climatic conditions and technological measures of growing crops.

According to our research, the lowest yield of *Camelina sativa* seeds of variety Hirsky, as it was expected, has been noted in the variant without fertilizers (control) where it averaged 1.03 $t.ha^{-1}$ in 2018–2021 (Fig. 1).

Increasing the dose of mineral fertilizers to $N_{30}P_{45}K_{45}$ provided yield increase – by 0.75 $t.ha^{-1}$, or 69.9%. Further increase in doses of mineral fertilizers also contributed to increase of crop productivity. Thus, in the variant with

application of mineral fertilizers at the dose of $N_{30}P_{45}K_{45}$+ N_{60} the crop yield increased to 2.04 t.ha^{-1}, which is by 1.01 t.ha^{-1} higher than the control, and by 0.26 t.ha^{-1} higher compared to the variant with $N_{30}P_{45}K_{45}$. On the fifth variant application of fertilizers in a dose of $N_{30}P_{45}K_{45}$ into main fertilization and in combination with micro–fertilizers and growth stimulants contributed to yield increase to 1.99 t.ha^{-1}, which is higher than the control by 0.96 t.ha^{-1}, and compared to the third variant ($N_{30}P_{45}K_{45}$) the yield increased by 0.21 t.ha^{-1}.

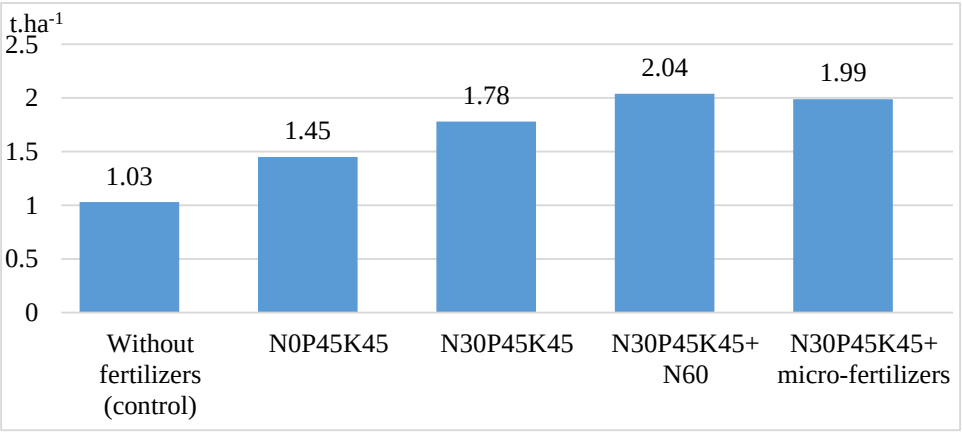

Figure 1. Yielding capacity of *Camelina sativa* depending on the level of mineral nutrition, t.ha^{-1} (2018–2021)

Mathematical models have also been created that describe the relationship between spring rye yield and a set of structural indicators. These models make it possible to predict the yield level of a crop using the number of pods, the number of seeds in a pod in one equation.

In order to establish relation between yielding capacity and structure indices, we analyzed three–year experimental data (2018–2021) and created mathematical models based on correlation–regression analysis that are reliable at 95% probability according to Fisher's and Student's criteria (Table 2).

Table 2. Mathematical model of relation between yielding capacity and indices of *Camelina sativa* structure, average for 2018–2021

Regression equation	Multiple correlation coefficient, R	Determination coefficient, D, %
$Y = 0.4805–0.0305X_1+0,0031X_1^2$	**0.967**	**93.5**

Note: Y – yielding capacity, t.ha^{-1}; X_1 – number of pods, pcs.; X_2 – number of seeds per pod, pcs.; X_3 – field germination of seeds, %.

The average and close correlation of yielding capacity with the number of pods and the number of seeds per pod was established. It is evidenced by coefficients of correlation (R = 0.967) and determination (D = 93.5).

It should be noted that yielding capacity calculated by the number of pods was the closest to actual, and by the number of seeds per pod had some differences. And calculated yielding capacity in terms of field germination was almost the same in all variants of the experiment, which confirms the idea about weaker relation between these indices.

CONCLUSIONS

The study of obtained data allowed to establish that the decisive role in the formation of *Camelina sativa* seed yield belongs to the use of mineral fertilizers in main phases of the crop ontogenesis. On average for 3 years of research, the increase in yield from application of mineral fertilizers and micro–fertilizers was 0.42–1.01 $t.ha^{-1}$, and combination of this measure with foliar fertilization of micro–fertilizers three times during vegetation period generated seed yielding capacity in amount of 1.99 $t.ha^{-1}$.

It has been found that the level of yielding capacity is determined by the following elements of the structure: the number of branches on the plant, the number of pods, the number of seeds in them and the weight of 1000 seeds. It has been established that all these indices increase significantly under influence of mineral fertilizers.

Mathematical models created on the basis of experimental data (2018–2021) reflect peculiarities of dependence between these indices. Based on the analysis of relation between yielding capacity and individual indices of the structure of *Camelina sativa*, it has been determined that the closest relation was between yielding capacity and the number of pods (R = 0.967). Therefore, the most accurately prediction of yielding capacity level of *Camelina sativa* according to calculated regression equation can be done by the number of pods.

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MIGRATION WAWES AND STAGE OF PIGMENTATION OF GLASS EELS FROM RIVER BOJANA (MONTENEGRO)

SUMMARY

The endangered European eel biological life cycle consists of five stages of metamorphosis: leptocephalus, glass eel, elver, yellow eel, and silver eel. Montenegro has limited data on biological and population parameters of glass eel e.g. accurate time and the number of migration waves, condition, rate of survival, recruitment success, etc. Since the early 1980s, the occurrence of glass eels in European waters has decreased significantly. Accordingly, after almost 25 years the first research was conducted on glass eel in the river Bojana (Montenegro). This study was done during the winter and spring of 2021 and 2022. Stage of pigmentation and condition were examined on 50 registered individuals of glass eel. Four pigmentation stages: VIA_0, VIA_1, VIA_2, and VII were registered. Its biometric characteristics indicate that one migration wave was registered with the domination of stage VIA_1. This research indicates the necessity of further research on the glass eel, as well as the threats they are facing in the river Bojana and another small river in the Adriatic catchment of Montenegro, with the aim of establishing the management plan for better protection conservation of this important but vulnerable species.

Keywords: European eel, Adriatic catchment, pigmentary stage, condition, migration

INTRODUCTION

The panmictic and catadromous European eel, *Anguilla anguilla* (Linnaeus, 1758) was listed as critically endangered (CR) on the IUCN Red List of Threatened Species in 2008, and remained in the same category by the most recent assessment published in 2020 (Freyhof & Kottelat, 2010; Pike *et al*., 2020). The endangered European eel biological life cycle consists of five stages of metamorphosis: leptocephalus, glass eel, elver, yellow eel, and silver eel. After hatching in the Sargasso Sea, eel leptocephalus larvae drift with the gulf stream

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(Tesch, 1977) until they reach the continental shelf where the larvae metamorphose into glass eels and migrate up the estuaries and colonize freshwater habitats. The glass eels sense the Earth's magnetic field and use it as a reference compass mechanism to orient (Cresci *et al*., 2017), as well as glass eels at sea swimming oriented towards the azimuth of the moon at the new moon when the moon rose above the horizon and was invisible but not during the other moon phases (Cresci *et al*., 2019). The duration of this migration is still controversial and could range from 7 to 9 months or at least 2 years (Lecompte-Finiger, 1994; Kettle & Hainse, 2006).

In the Adriatic Basin of Montenegro, the European eel occurs in all rivers draining into the Adriatic Sea. Despite their globally and commercial importance the data on the European eels in the Montenegrin coastal region are scarce and mainly concerned with the silver stage (Milošević & Mrdak, 2016; Rakočević *et al*., 2018; Kanjuh *et al*., 2018; Milošević *et al*., 2021; Marić *et al*., 2022). The first and only published data about the characteristics of glass eel migration in Montenegro based on research at the mouth of river Bojana that has been carried out from 1998-2002 was published by Hegediš *et al*., 2005. The Bojana River with the neighboring Porta Milena wetland complex and Lake Šasko represent the hydrologically largest and the most biologically complex aquatic unit in the entire coastal zone of Montenegro and important habitat for the overall stock of European eel in Montenegro.

This study had the following objectives: (1) to establish the number, time, and duration of migration waves of glass eel, and (2) to determine the morphometric characteristics and condition of presented pigmentary stages. The objectives are set in a way to check the parameters that were mentioned after almost 25 years of the latest survey.

MATERIAL AND METHODS

Study area. Bojana River is a 41 km long river situated in the southern part of the Montenegrin coast. It is an outflow of Lake Skadar and flows into the Adriatic Sea, forming the border with Albania in some extent. By its water amount brought into the sea, it is the third river on the Mediterranean (after the river Nile and Po in Italy). The source of the Bojana River lies at the southern part of Skadar Lake, the largest lake in southeastern Europe. Due to the deposition of the sediment conveyed by the Bojana River into the Adriatic Sea for centuries the Ada Bojana island was formed and today presents a unique ecosystem in Europe (Gršić *et al*., 2018; Petković & Sekulić, 2019).

Sampling. Research on glass eel on river Bojana was conducted in 2021 (from February to June) and 2022 (from February to May) (Figure 1).

Glass eels were gathered with the help of a handmade fyke net trap with wings and two funnels (three chambers). Traps were made of aluminum profile and tulle according to Hegediš *et al*., 2005 (Figure 2a). They were set on the right side of the river, at the depth of 60 cm (locations I and II, Figure 1) and on the small stream that flows into Bojana (location III, Figure 1). The surface of the

front entering part of the trap was 1.2 m^2. Traps were being emptied two times a week.

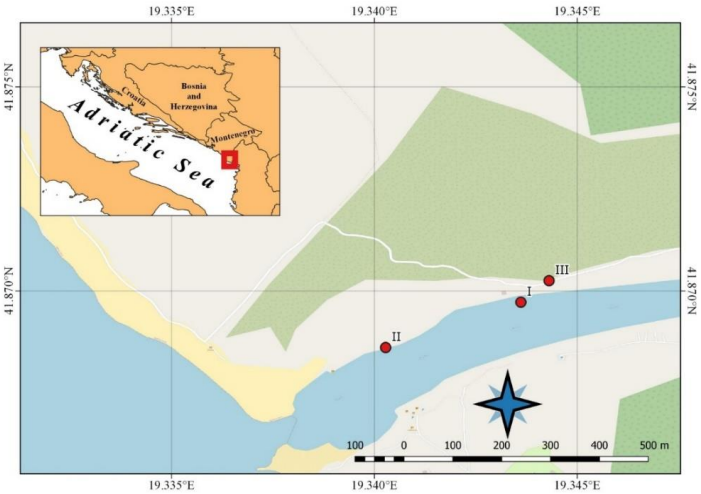

Figure 1. River Bojana and sampling locations of glass eel

The captured glass eel individuals were measured to the nearest 1 mm (total length, TL) and weighed to the nearest 0.1 g (weight, W), and then returned back to the water (Figure 2b). Stadium of pigmentation was determined according to Elie *et al*., 1982. Data of the Fulton's condition factor (CF) of the analyzed specimens was calculated from the W (g) and TL (cm) using the formula according to Fulton (1904).

Figure 2a (left). Fyke net at the sampling locations; **Figure 2b (right).** Glass eels from River Bojana (Montenegro). After measuring the glass eels were returned back to the water

Since the number of registered individuals in 2021 did not meet the statistically valid number of individuals for making relevant conclusions about their condition and quality, their basic biometric characteristics will be presented since these are the first findings and descriptions of these individuals after almost 25 years, and a more detailed analysis was done on a sample from 2022.

RESULTS

During the three-month survey in 2021, 10 glass eel individuals were recorded. The first individuals were registered on March 12, 2021. while the last ones were caught on April 5, 2021. During the months of April and May (until the beginning of June), not a single glass eel was registered in the catch. The average TL of individuals registered during 2021 was 8.8 cm ±2.52. The minimum recorded value of TL was 5.9 cm, while the maximum registered TL was 11.3 cm. The average W was 1.12 gr ±1.01. The minimum recorded value of W was 0.1 gr, while the maximum registered W was 2.1 gr.

In the second year of the research, 40 glass eel individuals were recorded. The first individuals were registered in the first week of February, while the largest number of individuals was registered from the end of February to the middle of March.

Table 1. The summary of descriptive statistics of observed pigmentary stages of glass eels during 2022 (N-number of individuals, minimum, maximum, and average values of total length (TL in cm) and weight (W in gr), condition factor (CF)

Pigmentary stage	*N*	*TL (min-max; average)*	*W (min-max; average)*	*CF*
VI $_{A0}$	15	5.5-6.6; 6.02	0.1-0.2; 0.12	0.83
VI $_{A1}$	18	5.8-6.9; 6.22	0.1-0.2; 0.12	0.75
VI $_{A2}$	5	5.8-6.6; 6.2	0.1-0.2; 0.12	0.72
VII	2	8.1-9.1; 8.6	0.8-1.5; 1.15	1.34

During the research conducted in 2021, two pigment stages were recorded in registered individuals: VI A_1 and VII. In the second year of the research, four pigment stages were registered: VIA_0, VIA1, VIA_2, and VII. The descriptive statistics of observed pigmentary stages during 2022 sampling are given in Table 1. The analysis of the contributions of registered pigment stages during 2021 showed that the dominant stage was VI $A1_1$ with 70%, while stage VII was represented with 30%. In the second year of the research, the largest number of registered individuals belonged to stages VI A_1 and VI Ao (45% and 37.5%), while the other two stages (VI A_2 and VII) were significantly less represented (13% and 5% respectively). According to biometric characteristics of pigmentary stages, one migration wave was registered with the domination of stage VIA_1.

The average value of the condition factor during 2022 was 0.82 with a range of minimum and maximum values of 0.39-1.55. The relationship between the condition factor and pigmentary stages registered during the observed migration wave in 2022 is given in Figure 3. The general trend shows that individuals with a lower pigment stage have higher values of CF while the increase of CF was observed in stage VII, again.

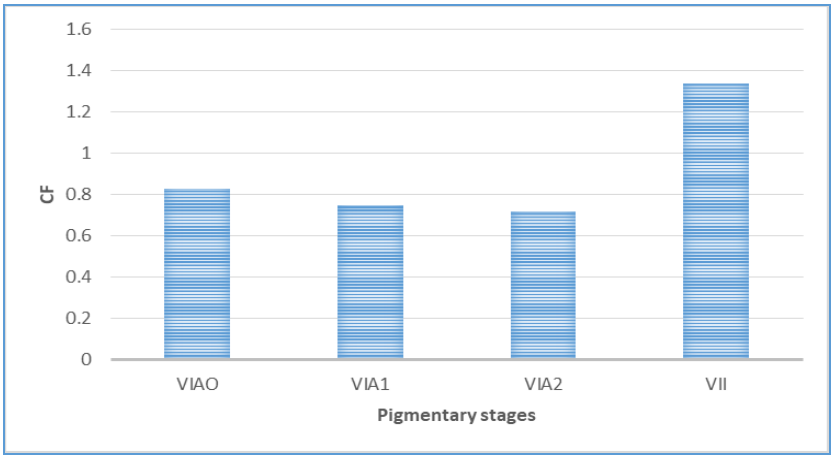

Figure 3. Condition factor of pigmentary stage of glass eel during migration wave in 2022.

DISCUSSION

The catadromous European eel *A. anguilla* reproduces in the Sargasso Sea from March to July (Schmidt, 1922; McCleave *et al*., 1998). In Europe, glass eels enter estuaries mostly from October to April/May (Elie, 1979). Since Montenegro has limited data on biological and population parameters of glass eels for almost 25 years the first research was conducted on glass eels in the river Bojana (Montenegro). It was established that the river Bojana can represent a significant source of high-quality glass eel, which can produce between 320 and 427 kg annually (Hegediš *et al*., 2005).

This study was done during the winter and spring of 2021 and 2022 i.e. from February to May. During the three-month survey in 2021, the first individuals were registered on March 12, 2021. while the last ones were caught on April 5, 2021. In the second year of the research, the first individuals were registered in the first week of February, while the largest number of individuals was registered from the end of February to the middle of March. In studies conducted 25 years ago in the same river, three migration waves were shown (Hegediš *et al*., 2005). The first wave occurred at the end of February and the beginning of March. The second wave occurred at the end of March and the beginning of April, while the third migration wave occurred at the end of April (Hegediš *et al*., 2005). Although the research presented in this paper covered the same research period, our research did not record the other two migration waves. It should not be overlooked that in the cited literature data, the sampling was carried out over a period of five years (cumulatively observed). In addition, the fact that the conditions on the Bojana River have changed significantly in the previous 24 years since the last survey was conducted. Two disastrous floods in 2009–2011 have significantly altered the bifurcation of the Bojana River, by forming a barrier of tree trunks and sediment bars in the right river reach. The civil engineering solution for restoring the flow capacity, based on dredging sand

bars, barrier removal, and bank consolidation, is opposed by environmentalists on the ground that these works are detrimental to the unique ecosystem (Petković & Sekulić, 2019). On the other hand as a result of global warming, the Sub Tropical Gyre in the Sargasso Sea inhibits thermocline overturn in the spring (Knights, 2003). Also, the sea warming in the eel spawning area since the early 1980s has modified marine production and affected the survival rate of European eels in early life (Bonhommeau *et al*., 2008) which consequently decrease larval growth and survival. Results of the study Borges *et al*., 2019 suggest a reduction in glass eel survival under a 4°C temperature increase. Decreased survival likely reflects lower body condition, which is considered a major cause for eels not to venture to full riverward migration (Edeline *et al*., 2006; Edeline *et al*., 2009). Thus, less amount of lipids would be available for glass eel energy stores during metamorphosis and this could lead to an increase in the estuarine settlement process (Bureau Du Colombier *et al*., 2007).

During the research conducted in 2021, two pigment stages were recorded in registered individuals: VI A_1 (70%) and VII (30%). In the second year of the research, four pigment stages were registered: VIA_0, VIA_1, VIA_2, and VII. In the second year of the research, the largest number of registered individuals belonged to stages VI A_1 and VI Ao (45% and 37.5%), while the other two stages (VI A_2 and VII) were significantly less represented (13% and 5% respectively). According to biometric characteristics of pigmentary stages, one migration wave was registered with the domination of stage VIA_1. The obtained results of this research somewhat coincide with the research of Hegediš *et al*., 2005. Namely, during the first migration wave (February - March), stage VI A_1 was dominant, which is in agreement with our results. Apart from this stage, stage VIA_0 was represented in a significant percentage. It is interesting that stage VII was not registered in the research of Hegediš *et al*., 2005, while during our research it was registered on two occasions, on 12.03. 2021 and 14-22.02.2022. Although the appearance of the final stage of pigmentation (VII) is reported in the literature for the third migration wave (April - May), our research showed the presence of these individuals in both years of sampling, in February and March, which indicates that the monitoring of migration waves should begin and before at the latest in December. Different authors give preference to different environmental parameters during the determination of the temporal and spatial profile of the migration. The most frequently associated factor is the tidal regime combined with the changes in water temperature, tidal regime, lunar cycle, and other environmental factors (Hegediš *et al*., 2005). Also, migration can be influenced by internal as well as environmental factors such as estuarine pollution, and temperature and salinity fluctuations. Relationships between CF and pigmentary stages show that individuals with a lower pigment stage have higher values. Further from stage VII, a significant increase in this factor is observed, which is in agreement with the fact that glass eels are fed exogenously at stage VII, which certainly leads to changes in body proportions and explains this trend in the value of the condition factor.

CONCLUSION

Taking in mind the surface of the inland Skadar Lake-Drim-Ohrid Lake system, delta of River Bojana is one of the most important area for the eel population in this part of the north-western Mediterranean. The presented result provide important data which, once again, confirms that delta of the Bojana river is an important nursery area for the young eels where this fish not only enter into the Skadar Lake- Drim-Ohrid Lake system but also implies the fact that this is the place where young eels undergo metamorphosis to the stage on which it starts with active feeding.

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A NOVEL TREATMENT FOR DETERMINING THERMAL CONDUCTIVITY OF THE SOIL SUBSTRATES FOR SELECTING SUSTAINABLE GROWING MEDIUMS IN TERMS OF THERMAL RESISTANCE

SUMMARY

The Thermal conductivity of the soil is important to determine the horticultural performance of growing medium. Microclimatic features influence the viability of soil mixtures for vegetation. The goal of this study is to assess the heat conductivity of soil mixture samples comprising Coir, Biochar, Sawdust, Wood bark, and Compost in order to discover the best growth medium in terms of thermal resistance. Specimens were prepared by mixing 60% of raw materials with 40% topsoil and moulded into cylinders. Each sample were converted into semi solids and undergone for thermal conductivity apparatus measurements. Thermal conductivity of each specimen was determined using mathematical analysis based on experimental readings. The calculated results were used to deduce thermal resistance. Thermal conductivity values of all specimens were observed in the range from 0.64 W/mK to 0.91 W/mK. The maximum and minimum thermal conductivity magnitudes were exhibited by Sawdust (60:40) and Wood bark (60:40) respectively. In terms of suitability as a growing medium, Wood bark (60:40) contains highest thermal resistance while the lease thermal resistance was determined in Sawdust (60:40) due to its high thermal conduction. This research concludes that Wood bark (60:40) is the most convincing substrate in terms of thermal sustainability.

Keywords: Growing medium, Thermal resistivity, Thermal Conductivity Apparatus, Microclimatic features, urban ecosystem

INTRODUCTION

Soil is one of the most important and most complex natural resources, but current developments (climate change, soil erosion, and urbanization) increasingly threaten this valuable resource (Spalevic *et al.*, 2020). According to the United States Department of Agriculture soil is defined as a natural body

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comprised of solids (minerals and organic matter), liquid, and gases that occurs on the land surface, occupies space, and is characterized by one or both of the following: horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment (Spalevic, 2011; Santana *et al.*, 2021; Spalevic *et al.*, 2020; Rodrigues Neto *et al.*, 2022). Plant substrate is defined as a substance on or in which plants grow. Thus, even soil can be viewed as a substrate. The main functions of the substrate are generally: providing plant anchorage, and delivering water, nutrients and oxygen to the roots.

Thermal property of aggregates is a primitive requirement in many fields like engineering, materials science and agriculture. Recent studies have shown that there is an increasing trend of research activities to find thermal properties. According to (Ghuman and Lal., 1985) the microclimatic features are influenced by thermal properties of soil, most particularly the stand establishment, germination of crops and the emergence. Thermal conductivities of the soil substrates are determined by elements that may be classified into two categories: inherent characteristics and externally manageable ones. Soil texture and the mineral content can be categorized into inherent properties (Wierenga *et al.*, 1969) while soil management and moisture content can be included into the externally manageable factors. Among the manageable factors, moisture content is the most difficult parameter to manage (Yadav and Saxena, 1973). In the early studies (Riha *et al.*, 1980), special consideration was given to studying the effect of water content on the thermal conductivity of soil aggregates, and it was discovered that the maximum state of soil conductivity is observed during the moist state of soil because the flow of mineral ions facilitates more thermal conduction than the dry state of soil. Studies conducted by Parikh *et al.*, (1979) and Noborio & McInnes (1983) have revealed a decrease in thermal conductivity along with the increased concentration of mineral salts such as $CaCl_2$, $MgCl_2$, NaCl and Na_2SO_4 especially in 0.1 mol/kg of heterogeneous soil solution. Thermal conductivity of water moistened Quartz sand was comparatively higher than the thermal conductivity of same type of Quartz sand moistened with 0.25 mol/kg KOH (Globus & Rozenshtok, 1989). Therefore, the study has been conducted with moistened substrate specimens to study the thermal conductivity behaviour without discrepancy.

The trial approach to measure the thermal conductivity of soil by using temperature rise or fall was first developed in the studies based on soil analysis (Jackson & Taylor, 1965) and the method was further developed with volumetric heat capacity of soil from volumetric proportions in accordance to shape of the soil aggregates (De Vries, 1963). One of the milestones in thermal conductivity studies was the discovery of Dual probe technique (Bristow *et al.*, 1993; Kluitenberg *et al.*, 1993). In this method, two needle probes in parallel are placed; one contains a heater and the other a temperature sensor. Dual probe technique was used in the studies conducted by Sailor *et al.* (2008) under ASTM D5334 guidelines to measure the thermal conductivity of soil mixtures. However, the needle probe method is a relatively expensive way and it requires certain expertise in using the microcontrollers and sensors. Therefore, it is not always a feasible option in the developing countries, where the artificial intelligence

programming technology is still not in use widely. Hence, the entire information about the corresponding soil specimen needs to be completely known to use it as an input data. Furthermore, this method is entirely based on the programming of sensors, thus resulting in an unpredictability of errors in measurements if a lack of skills in programming were to prevail.

Lee's apparatus is effective in finding the thermal conductivity of weak conductors, it is not appropriate in this circumstance since it is difficult to mould wood bark and Biochar specimens into a uniform disc shape. Although if moulding were possible, the non-uniformity in aggregate distribution of the five selected specimens would not be a suitable specimen state for performing Lee's disc technique according to the outcomes of past research on gelatine-silica aerogel, the heterogeneous mixtures cannot be moulded as discs. Therefore, study is designed to find the thermal conductivity of five different substrate mediums and to ultimately study their thermal resistance behaviours for comparative analysis using the customized thermal conductivity apparatus. The objective is to assess the heat conductivity of soil mixture samples comprising Coir, Biochar, Sawdust, Wood bark, and Compost in order to discover the best growth medium in terms of thermal resistance.

MATERIAL AND METHODS

Theory. Heat can be transferred by three different methods like conduction, convection and radiation. Each method can be analyzed by in its corresponding mathematical principles. Total heat transfer through a material in terms of the material's thermal conductivity (k) Surface area of conduction (A); temperature gradient (T); material thickness (H); heat transfer (q); and time of conduction (t) is:

$$\Delta q = \frac{\Delta T k A \Delta t}{H}$$

Therefore, thermal conductivity $k = \frac{H \Delta Q}{\Delta t A \Delta T}$ in (in cal/cm.sec.℃)

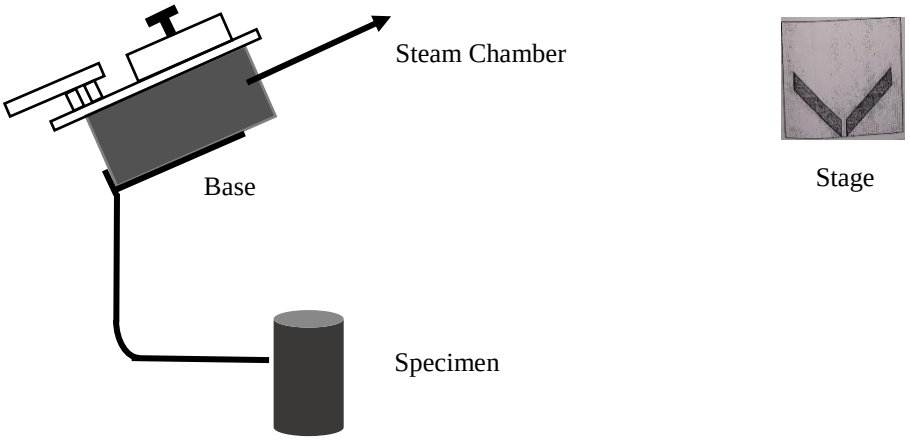

Figure 1. Thermal conductivity

Thermal resistance (R) could be derived as $R = \frac{\Delta x}{kA}$

Sampling and Analysis. The specimens were prepared at the Faculty of Applied Science, South Eastern University of Sri Lanka (SEUSL) in May 2021 by using the five growing mediums namely Biochar, Coir, Sawdust, Wood bark and Compost. 60% of each specimen was mixed with 40% of topsoil. The prepared samples were compacted with 35 blows under ASTM standards and moulded cylindrical shapes. Afterwards, each moulds were converted into semi solid mixtures through mixing with water under 1:2 (w/w) according to ASTM D70 guidelines. The experiment was carried out in open air at room temperature. Specimens were put on stage so that they could move freely. Prior to evaluating each specimen, the temperature of the steam chamber was monitored. Because the testing laboratory was not located at the Mean Sea Level, it cannot be assured that the equipment is at 100°C. Following that, heat is allowed to circulate through the sample. The total time were measured immediately when the first sight of the melting from specimen was observed. Melting of specimen was allowed to gather in the beaker for twenty minutes and afterwards heat transmission stopped. The mass and temperature of the specimens in the beaker were measured and afterwards entered into the equations. The experiment was conducted for three days. Each specimen was undergone with experimental activities three times under same steps and the corresponding thermal conductivity values were measured. Ultimately, the averaged results were reported, and the thermal resistances of specimens were measured. The determined values were translated into SI units. Table 1: Characteristics of the diary manure based on batch hydrolysis

RESULTS AND DISCUSSION

Thermal conductivity of five chosen substrate specimens are as follows: Biochar, Coir, Sawdust, Wood bark, and Compost amended with soil. Table 1, Table 2, and Table 3 show the computed findings. The results are influenced by external factors such as changes in room temperature, changes in air pressure, and the possibility of human error. As a result, the mean values were computed.

Table 1: Thermal Conductivity results for day 01

Day 01 **Substrate**	**Weight of collected substrate + Beaker (g)**	**Weight of Beaker (g)**	**Weight of substrate**	**Collection time (s)**	**H (cm)**	**T2 - T1**	**Inner diameter (cm)**	**A(cm^2)**	**Thermal Conductivity (W/m.K)**
Biochar (60:40)	57.61	43.28	14.33	1200	2.1	38.3	6.35	31.67	0.69
Coir (60:40)	57.72	42.48	15.24	1200	2.17	38.1	6.35	31.67	0.76
Sawdust (60:40)	60.88	42.45	18.43	1200	2.22	38.4	6.53	33.49	0.89
Wood bark (60:40)	56.16	42.45	13.71	1200	2.08	38.9	6.53	33.49	0.61
Compost (60:40)	57.58	43.28	14.3	1200	2.1	37.6	6.35	31.67	0.70

Table 2: Thermal Conductivity results for day 02

Day 02 Substrate	Weight of collected substrate + Beaker (g)	Weight of Beaker (g)	Weight of substrate	Collection time (s)	H (cm)	T2 - T1 (℃)	Inner diameter (cm)	A(cm^2)	Thermal Conductivity (W/m.K)
Biochar (60:40)	58.33	43.28	15.05	1200	2.1	38.3	6.35	31.67	0.73
Coir (60:40)	57.47	42.48	14.99	1200	2.2	38.2	6.35	31.67	0.76
Sawdust (60:40)	61.89	42.45	19.44	1200	2.19	38.7	6.53	33.49	0.92
Wood bark	56.4	42.45	13.95	1200	2.13	38.5	6.53	33.49	0.64
Compost (60:40)	58.01	43.28	14.73	1200	2.05	38.1	6.35	31.67	0.7

Table 3: Thermal Conductivity results for day 03

Day 03 Substrate	Weight of collected substrate + Beaker (g)	Weight of Beaker (g)	Weight of substrate	Collection time (s)	H (cm)	T2 - T1 (℃)	Inner diameter (cm)	A(cm^2)	Thermal Conductivity (W/m.K)
Biochar (60:40)	58.04	43.28	14.76	1200	2.06	38.6	6.35	31.67	0.69
Coir (60:40)	58.2	42.48	15.72	1200	2.13	37.9	6.35	31.67	0.78
Sawdust (60:40)	62.88	42.45	20.43	1200	2.1	38.6	6.53	33.49	0.93
Wood bark (60:40)	57.32	42.45	14.87	1200	2.04	38.7	6.53	33.49	0.65
Compost (60:40)	59.26	43.28	15.98	1200	2.12	37.7	6.35	31.67	0.79

Table 4: Mean Thermal conductivity values for test specimens

Substrate	Mean Thermal conductivity	Rank
Biochar (60:40)	0.70	4
Coir (60:40)	0.77	2
Sawdust (60:40)	0.91	1
Wood bark (60:40)	0.64	5
Compost (60:40)	0.73	3

Table 5: Moisture content of substrate specimens

Substrate medium	$W_{sample,sat}$(kg)	γ (kN/m^3)	$W_{sample,dry}$(kg)	γ_{dry}(kN/m^3)	w	Rank
Saw dust	0.409	3.212	0.239	1.874	0.71	1
Coir	0.557	4.370	0.350	2.748	0.59	2
Wood bark	0.554	4.349	0.361	2.835	0.53	3
Bio char	0.579	4.511	0.387	3.040	0.48	4
Food waste	0.657	5.152	0.509	3.994	0.29	5

Table 6: Computation for the average substrate thickness

	Substrate thickness (cm)			
Substrate	**h_1 (Day 01)**	**h_2 (Day 02)**	**h_3 (Day 03)**	**Δx *(cm)***
Biochar(60:40)	2.1	2.1	2.06	2.09
Coir(60:40)	2.17	2.2	2.13	2.17
Sawdust(60:40)	2.22	2.19	2.1	2.17
Wood bark(60:40)	2.08	2.13	2.04	2.08
Compost (60:40)	2.1	2.05	2.12	2.09

Table 7: Experimental outcomes of Thermal resistance

Substrate (60:40)	A (cm^2)	Δx *(cm)*	k (W/m.K)	Thermal resistance R_O (K/W)	Rank
Coir	31.67	2.17	0.77	8.91	**4**
Compost	31.67	2.09	0.73	9.06	**3**
Biochar	31.67	2.09	0.70	9.36	**2**
Wood bark	33.49	2.08	0.64	9.79	**1**
Sawdust	33.49	2.17	0.91	7.12	**5**

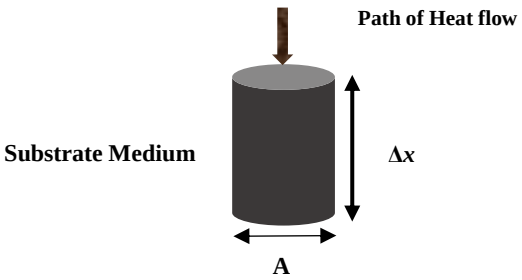

Figure 2: Heat flow across test specimen

Thermal conductivity results in Table 4 has shown that all the specimens exhibits thermal conductivity magnitudes corresponding to "sandy loam" (i.e. 0.64 $Wm^{-1}K^{-1}$ to 0.91 $Wm^{-1}K^{-1}$) soil group. According to a previous study soil thermal conductivity effects, sandy loam possess thermal conductivity from 0.19 $Wm^{-1}K^{-1}$ to 1.12 $Wm^{-1}K^{-1}$ (Abu-Hamdeh and Reeder, 2000; Abu-Hamdeh, 2003).) thus verifying the accuracy of the current experiment executed in this study. The largest thermal conductivity was found in sawdust (60:40), while the lowest magnitude was found in wood bark (60:40). The following part of the analytical investigation looked into the influence of water content reported by (Yadav and Saxena, 1973) on their experimental outcomes. Table 5 shows the calculations to study the effects of moisture content at thermal conductivity of our selected substrates. According to the findings of Table 4 and Table 5, the thermal conductivity of substrate is proportional to its water retaining ability and our study validates the outcomes of Yadav and Saxena (1973).

The primary goal for determining thermal conductivity was to identify the best substrate for heat resistance and thermal comfort for the sustainable agriculture. The heat flow scenario across substrate specimens can be effectively described by Figure 2. The thermal resistances of all five substrates were determined using the heat flow and the equation for computation, as shown in Table 7 where Wood bark (60:40) emerged as the best fitting substrate in terms of thermal resistance. To avoid the observational mistakes experienced while manipulating the Vernier, the average specimen thicknesses were evaluated. Furthermore, the results of Tables 4 and 5 indicated that thermal performance magnitudes of soil substrates could get externally manipulated via altering the moisture content of growing mediums. This finding supports the conclusions reached by Parikh *et al.* (1979); Yadav and Saxena (1973); Riha *et al.* (1980).

Since there were no prior thermal conductivity experiments for the stated substrate materials had been undertaken, the scientists were unable to compare the results to any earlier investigations. The complexity of the default mode of soil, the consistent organic content of each substrate specimen because of climate stability, as well as the volatility of bioactivity within soil are all reasons for the paucity of study in this topic.

CONCLUSION

The findings of this study reveal that heat conductivity varies according to organic content, soil texture and water content. Thermal conductivity increases as moisture content increases.

Furthermore, the study's findings are backed by the conclusion remarks of Abu-Hamdeh and Reeder (2000), which affirm that all types of soil correspond to the sandy loam category, which is excellent for horticulture and planting. It demonstrates that the test was done without mistakes because the selected growth media specimens were previously known before to the experiment that they belonged to the sandy loam type.

Finally, this experimental study on substrates made from organic wastes amended with soils concludes that, in terms of thermal resistance after studying the thermal conductivity behaviour of substrates, the most convincing growing medium would be Wood bark (60:40) and the least suitable would be Sawdust (60:40).

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BIOMETRIC CHARACTERISTICS AND HEALTH STATE OF ENGLISH OAK (*QUERCUS ROBUR* L.) STANDS ESTABLISHED USING VARIOUS STOCK TYPES

SUMMARY

Planting methods and stock quality significantly influence productivity and resilience of future stands. However, there is no consensus on planting practices and stock types to grow resistant English oak (*Quercus robur* L.) stands in various site conditions. In the study, we investigated English oak forest stands established in 2010. The study area is situated in forest-steppe zone (Kharkiv region, Ukraine). The stands were established on a non-uprooted felling site using different methods and stock types: planting bare-root and containerized seedlings and direct sowing of acorns. The highest average height and diameter were registered in 11-year-old oak stand established with container seedlings. The differences in height and diameter were statistically insignificant compared with the stand established by sowing and bare-root seedlings. The best health condition was identified for the direct-seeded stand. The oak height increment intensity in the direct-seeded stand, estimated from the average current height increment, was 2.8% and 5.5% higher than in the stands grown from container and bare-root seedlings, respectively. Direct sowing should be preferred for artificial establishment of oak stands in fresh rich forest sites within the forest-steppe zone in the case sufficient crop of acorns is available no threatened with rodents or wild boars damage. This method is inexpensive and best suited to forest nature. Planting bare-root and containerized seedlings is also relevant in the absence of a sufficient number of germinative acorns for sowing.

Keywords: acorn sowing; bare-root seedlings; container seedlings; current height increment; young stands

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INTRODUCTION

English oak (*Quercus robur* L.) is one of the most common tree species in Ukrainian forests. Oak stands occupy 28% of the total forest area of the country (2.7 million hectares) (Tkach *et al.*, 2019). Oak forest management aims at the cultivation of resilient, highly-productive and long-lived stands.

In Ukrainian forests, oak regeneration occurs both naturally and artificially. The latter way has an advantage due to the periodicity of fruiting of English oak, which is an average of five years (Majboroda, 2010; Prévosto *et al.*, 2015). Artificial regeneration of oak stands is done in two ways, by sowing acorns and planting bare-root or containerized seedlings. Both methods have pros and cons (Thadani, 2008; Zadworny *et al.*, 2014; Prévosto *et al.*, 2015; Löf *et al.*, 2019; Yavorovskiy and Segeda, 2019).

The method of establishing oak stands as well as the stock type and quality influence the productivity, health and resistance of future forests to the adverse impacts of various biotic and abiotic factors.

In Central Europe, due to more favourable climatic conditions, forest management is focused on the regeneration of oak stands by natural seeding or by sowing acorns. At the same time, many studies (Arosa *et al.*, 2015*; Ivetić et al.*, 2016; Stojanović *et al.*, 2017; Grossnickle and MacDonald, 2018) are devoted to the issue of sustainable forest management, which emphasize the need to follow all the prescribed technological processes and operations during artificial reforestation, which are strictly regulated by terms and quality.

Currently, there is no consensus among researchers regarding the methods of establishing artificial oak stands and stock type. Some (Madsen and Löf, 2005; Thadani, 2008; Zadworny *et al.*, 2014; Prévosto *et al.*, 2015; Ostapchuk *et al.*, 2018; Löf *et al.*, 2019) give preference to sowing acorns as a simple, inexpensive and most natural way of regeneration, while others (Hahn, 1982; Bondar and Hordiienko, 2006; Dey *et al.*, 2007; Tarnopilsky *et al.*, 2019; Yavorovskiy and Segeda, 2019) prioritize planting bare-root or containerized seedlings.

This issue is extremely relevant to countries whose oak forests occupy large areas. Proof of that is the study of a research team including scientists from many European countries (Leverkus *et al.*, 2021). In the study, the specificities of oak forests regeneration using various methods have been investigated throughout the European continent, including sowing acorns and planting seedlings.

The aim of the study was to compare biometric characteristics (diameter and height) and the health condition of English oak stands established using different methods and stock types: container and bare-root seedlings and direct sowing of acorns.

MATERIAL AND METHODS

The research was conducted in the oak forest site in north-eastern part of Ukraine (Kharkiv region), within the forest-steppe zone. In the region, deciduous forests predominate, with English oak as the most common forest-forming species. The area of oak-dominated forests is more than 84% of the total forest

area. The climate of the study region is temperate continental. The average air temperature varies from + 21 °C in the summer to -7 °C in the winter. The growing season is on average 190 ± 5 days. The average annual rainfall is 492 mm, of which 280 mm falls in the growth season. The height of the snow cover is 18–28 cm (Ecological passport of Kharkiv region, 2021).

Soils are mainly light-textured loam and clay chernozems (Ecological passport of Kharkiv region 2021).

Geographical coordinates of the study site were the following: 50°03′54.0″ N; 36°06′09.0″ E. Growth specificities of planted oaks were studied in three plots with an area of 0.3 hectares each. Acorns were sown in the autumn of 2009; the stands were established in the spring of 2010, after a strip-gradual regeneration felling with 25 m wide felled strips. In the Plot 1 (Acorn treatment), two or three acorns were sown in each seed spot, at spacing of 4.0 × 0.7 m. In the experiment, acorns harvested in the study region were used for planting. Plot 2 (Container treatment) was established by planting one-year-old containerized seedlings with powered hole borer at 4.0 × 1.0 m spacing. In the Plot 3 (Bare-root treatment), oaks were established by planting one-year-old bare-root seedlings using Kolesov's planting iron, at 4.0 × 0.7 m spacing. In all plots, soil was treated in strips with a plough. Weed removal in rows was carried out three times during the first year after the stand establishing, three times in the second year, two times in the third year and once in the fourth year. Shrubs and secondary tree species were removed in space between the rows at the age of three; at the age of seven, a liberation felling with a Stihl brush cutter was made in the stands. The felling was carried out equally and simultaneously in all three plots, so this factor did not have a significant impact on the oak condition. During the liberation felling at the age of seven, one best oak plant was left in each seed spot in Acorn treatment.

Oak trees were surveyed in rows on each plot. In each row, the survey was conducted for 20 linear meters along the diagonal of the plot. The total length of the surveyed rows was 100 m where 100–120 oak trees were measured. The height of the oaks was measured with an accuracy of 1 cm with a special telescopic levelling stuff. Tree diameter was measured with an accuracy of 0.1 cm at a height of 1.3 m using Intertool MT-3015 calliper. The survival of oaks was estimated as the percentage of living oak trees from the number of planted ones.

The current height increment Z (cm) was determined by the formula (1):

$$Z = H_A - H_{A-1} \quad (1)$$

where:

H_A = the stand height in the study year (cm);

H_{A-1} = the stand height in the previous year (cm).

To be able to assess and compare height increment intensity of the stands having different initial parameters (age and average height), we calculated the average current height increment Z_{AVE} (cm) for the relevant observation period by the formula (2):

$$Z_{AVE} = \frac{Z_1+Z_2+\cdots+Z_n}{A} \quad (2)$$

where:

Z_n = current height increment of the relevant year (cm);

A = age of the stand (years).

The height increment intensity was defined as the relative difference in the current height increments between treatments, expressed as a percentage.

We assessed health condition of the trees using six categories (Table 1).

Table 1. Scale used to assess the stand health (Voron *et al*., 2011).

Health condition index range	Stand damage degree	Health status of the stand	Average health condition category
1.00−1.50	None	Healthy trees	1
1.51−2.50	Light	Weakened trees	2
2.51−3.50	Moderate	Severely weakened trees	3
3.51−4.50	Severe	Dying trees	4
4.51−5.00	Very severe	Standing dead trees died over the current year	5
5.01−6.00	Very severe	Standing dead trees died over recent years	6

Stand health condition was described by the health condition index I_c that can be obtained by the formula (3) (Voron *et al*., 2011):

$$I_c = \frac{K_1 n_1 + K_2 n_2 + \cdots \ldots + K_6 n_6}{N} \quad (3)$$

where:

$K_1 \ldots K_6$ = the category of the health condition of the trees (from 1 to 6);

$n_1 \ldots n_6$ = the number of trees of the given health condition category;

N = the total number of the recorded trees in the sample plot.

Normality tests, one-way analysis of variance (ANOVA), Tukey HSD test with a significance level of $p < 0.05$ were performed for data analyses.

The Box-Cox transformation was used to transform the data to the normal distribution, stabilize group variances and meet the homoscedasticity condition (Hammer *et al*., 2001). The Tukey's pairwise ANOVA (Hammer *et al*., 2001; Delacre *et al*., 2019) was used to find statistically significant differences between the statistical samples of height (Table 2), diameter (Table 3) and current height increment (Table 4) measurements.

RESULTS

Average height of 11-year-old oak stands in different treatments varied within 464–479 cm (Table 2). The height of the stand established with bare-root

seedlings was 2% and 3% lower than those established by direct sowing of acorns and using container seedlings, respectively**.**

Table 2. Average height (cm) of oak seedlings for different age and growth conditions.

Treatments	Age (years)						
	1	2	3	6	7	9	11
Acorn	13.37±0.57[a]	42.21±0.43[a]	62.74±2.72[a]	219.89±5.11[a]	269.38±6.35[a]	363.18±8.69[a]	474.21±6.42[a]
Container	26.48±0.86[b]	50.07±0.78[b]	69.16±2.22[b]	224.38±4.91[a]	274.38±8.28[a]	371.05±8.84[a]	478.97±5.95[a]
Bare-root (control)	21.84±0.58[c]	44.83±0.56[c]	60.70±2.52[a]	214.11±2.92[a]	264.38±5.72[a]	359.02±8.27[a]	464.12±7.95[a]
F	97.39	39.47	3.52	1.76	0.52	0.50	1.12
p	< 0.01	< 0.01	0.03	0.18	0.60	0.61	0.33

Values show the mean +/- standard deviation. Different letters in the columns (a, b, c) indicate significant difference between treatments ($p < 0.05$, Tukey's pairwise ANOVA).

The height dynamics in the investigated stands during 11 years of observations are noticeable when estimating their heights in relative values (Table 3). In the first year, direct-seeded oaks were significantly lower in height compared with those in the Bare-root (control) and Container treatments: by 41% and 50%, respectively. For the second year, the difference decreased considerably, to 7% and 16%, respectively, being statistically significant. As the root systems of trees established by sowing acorns had not been damaged during transplanting, the height difference gradually levelled off. Therefore, Table 3 shows that the height increment intensity for oaks in the Acorn treatment is higher than that in the Bare-root and Container treatments. The difference in average heights between the treatments and control decreases with age.

Table 3. English oak stand height in different treatments as a percentage from the control height (Bare-root).

Treatment	Age of stand (years)										
	1	2	3	4	5	6	7	8	9	10	11
Bare-root (control)	100	100	100	100	100	100	100	100	100	100	100
Acorn	59	93	103	103	103	103	102	101	101	101	102
Container	118	111	113	107	105	105	104	104	103	103	103

The oak stand established by sowing acorns had the smallest average diameter (Table 4). The stand established with bare-root seedlings had 5% larger diameter. The stand established with container seedlings had the largest average diameter of 9% greater than that of direct-seeded stand.

The significance of the differences between the average diameters in the Acorn and Container treatments as well as in the Acorn and Bare-root treatments up to the age of nine is explained by the fact that the stand established by sowing

acorns was dense before the liberation felling at the age of eight due to the presence of one to three oaks in the same seed spot.

Improvement felling and removal of inferior oak trees promoted intensive tree diameter growth. As a result, in 9–11-year-old stands, the differences between the average diameters in the Acorn and Container treatments and Acorn and Bare-root treatments decreased and became statistically insignificant.

Table 4. Average diameter (cm) of oak seedlings for different age and growth conditions.

Treatments	Age (years)			
	6	7	9	11
Acorn	0.98±0.04^{a}	1.46±0.07^{a}	2.72±0.12^{a}	3.90±1.22^{a}
Container	1.43±0.08^{b}	2.06±0.10^{b}	3.13±0.10^{b}	4.28±0.10^{b}
Bare-root (control)	1.43±0.05^{b}	1.96±0.07^{b}	3.03±0.12$^{a.b}$	4.14±0.10$^{a.b}$
F	25.55	18.15	3.57	2.94
p	< 0.01	< 0.01	0.03	0.05

Values show the mean +/- standard deviation. Different letters in the columns (a, b, c) indicate significant difference between treatments ($p < 0.05$, Tukey's pairwise ANOVA).

The lowest current height increment was observed in the oak stands established with seedlings (Container and Bare-root treatments) in the first year, in the so-called root-taking phase, which lasts one or two years. Instead, this variable in the Acorn treatment was significantly higher than in the Container and Bare-root treatments, by 38% and 54%, respectively, because the root systems of the direct-seeded trees were not damaged. A significant decrease in current increment occurred in 3-year-old stands in the dry growing season in 2012 (Table 5).

Table 5. Current height increment (cm) of oak seedlings for different age and growth conditions.

Treatments	Age (years)					
	1	2	3	4	5	6
Acorn	13.37±0.57^{a}	28.77±0.72^{a}	20.64±0.80^{a}	50.00±1.93^{a}	67.35±3.31^{a}	40.10±1.93^{a}
Container	7.65±0.32^{b}	25.53±0.62^{b}	18.60±0.66$^{a.b}$	48.68±1.75^{a}	65.10±3.37^{a}	41.30±2.97^{a}
Bare-root (control)	6.12±0.35^{c}	23.78±1.07^{b}	16.00±0.91^{b}	48.99±2.00^{a}	64.92±2.49^{a}	39.23±2.14^{a}
F	58.91	9.25	7.32	0.13	0.19	0.16
p	< 0.01	< 0.01	< 0.01	0.87	0.83	0.85

Values show the mean +/- standard deviation. Different letters in the columns (a, b, c) indicate significant difference between treatments ($p < 0.05$, Tukey's pairwise ANOVA).

The highest current growth was observed in 2014 in 5-year-old stands in all experimental treatments, with the maximum in the Acorn treatment (67 cm). In

this year, 318 mm of precipitation fell during April–August at a standard amount of 260 mm, i.e. the rainfall was 22% over the normal.

To assess and compare the height increment intensity for the stands with different initial values of age and height, the average current height increment over 11 years of observations was calculated. It was the largest in the Acorn treatment, amounting to 42.8 cm. The value was 2.8% above the Container treatment (41.6 cm) and 5.5% above the Bare-root treatment (40.5 cm).

The health condition of oaks from 2 to 11 years of age was assessed as healthy in all treatments (I_c was between 1.1 and 1.4). The stand condition in the Container and Bare-root treatments was rated as weakened (I_c was 1.6 and 1.7, respectively) only in the first year of establishment due to transplantation and adaptation of seedlings to new growing conditions in the regeneration site. Instead, the condition of the first-year direct-seeded stand was assessed as healthy. The best value of the average health condition index was noted in the Acorn treatment. The indicators were slightly worse in the Container and Bare-root treatments (Figure 1).

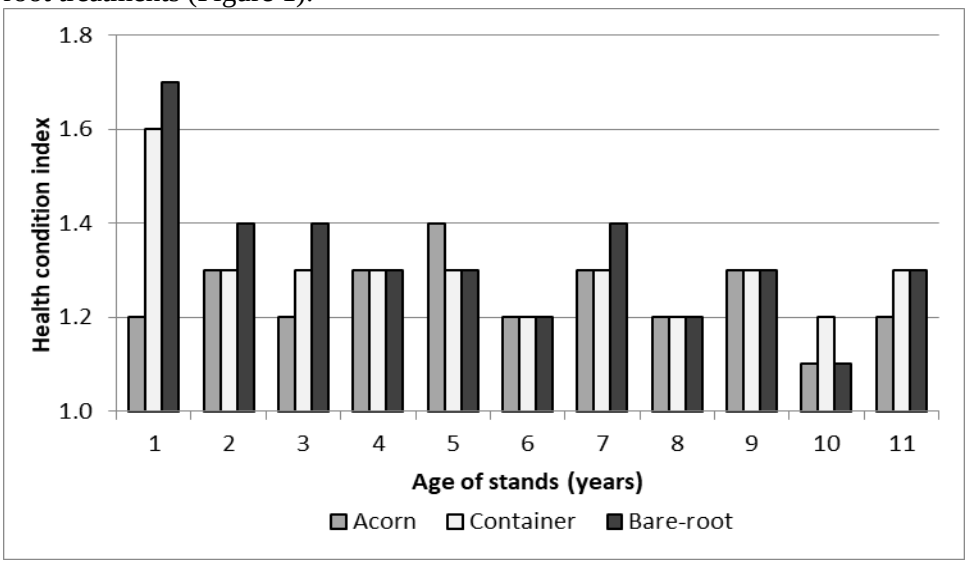

Figure 1. Health condition index of English oak stands.

At the age of seven years, before the liberation felling, the vitality of oaks was the highest (96%) in the treatment established with containerized seedlings. The value was slightly lower (85%) in the stand established with bare-root seedlings and the lowest (81%) in direct-seeded stand.

DISCUSSION

The reforestation technique and the stock type and quality influence the productivity and resistance of future forests to diseases and pests. At the same time, there are different approaches on the establishment of English oak stands in

various forest site conditions (Tovstukha *et al.*, 2017; Ostapchuk *et al.*, 2018; Yavorovskiy and Segeda, 2019).

According to Hordiienko *et al.* (2005) and Ostapchuk *et al.* (2018), direct sowing of acorns should be preferred for establishing oak stands in fresh and moist fertile sites. This reforestation method is simple and inexpensive; it is most consistent with the nature of a forest. The cost of sowing acorns is one third lower than the cost of planting seedlings. Such forest stands adapt better to environmental conditions. The root system of oak trees arisen from seeded acorns is not damaged, as there is no need for the transplanting (Hordiienko *et al.*, 2005; Thadani, 2008; Zadworny *et al.*, 2014; Prévosto *et al.*, 2015). Sown oak stands tolerate drought better than those established with seedlings (Zadworny *et al.*, 2014) due to the naturally developed taproot.

Seedlings provides some advantages over acorn sowing, among them evenly spaced seedlings. The seedlings are less suppressed by grass vegetation and undergrowth of other woody plants. The number of necessary interventions decreases and the crowns close faster in such stands established from seedlings (Bondar and Hordiienko, 2006).

Forest stands established with seedlings are less resistant to pests and diseases than those established by direct sowing of acorns because taproots were often damaged during transplantation from a nursery. Such trees are not good in self-pruning, which is then reflected in the technical quality of wood (Rudnev and Rybachok, 1975; Hensiruk, 2002).

One of the ways to increase forest regeneration efficiency is the establishment of forest stands using container-grown stock. This stock type has some advantages, among them the shorter root-taking time, more intense growth and development of plants after replanting on a regeneration site, high survival percentage as a result of minor damage to the roots during transplanting, and ability to plant trees during the entire growing season (Maurer, 2011; Tovstukha *et al.*, 2017; Tarnopilsky *et al.*, 2019; Yavorovskiy and Segeda, 2019).

Among the shortcomings for the cultivation of containerized oak seedlings are additional costs required for the container purchasing and the soil mixture preparing. Furthermore, the roots are often damaged when planting seedlings in the soil, thus weakening plants (Tarnopilskiy *et al.*, 2016; Yavorovskiy and Segeda, 2016). Lyalin (2013) investigating the economic efficiency of container and bare-root English oak seedlings cultivation, has found that containerized seedling growing is 40% more expensive.

Our data are consistent with the findings of Yavorovskiy and Segeda (2019) in Ukrainian Right-Bank Forest-Steppe. They have found that the average height and diameter of 8-year-old oak stands established with containerized seedlings exceeded those of stands established with bare-root seedlings and by sowing acorns. However, the increase for the containerized seedlings was more significant (23 and 43% in height and 20 and 44% in diameter, respectively) than in our study. Survival of container-grown seedlings at the end of the growing season was 98%, bare-root seedlings – 86% and by sowing acorns – 80%.

Instead, in our experiment, 11-year-old English oak stand in the Container treatment had slightly increased average height and diameter as compared with the Bare-root and Acorn treatments; the differences were only 3% and 1% over Bare-root treatment and 5% and 9% over the Acorn treatment, respectively.

Similar results were obtained during the study of forest stands established with container and bare-root stock at different planting densities in another permanent research object within Ukrainian Left-Bank Forest-Steppe (Tarnopilska and Korotych, 2018).

The study showed that 8-year-old oak stands established with container stock were higher than those of the same age established with bare-root seedlings in almost all treatments, regardless of density. The height difference was within 4–26% and the difference in average diameter at breast height was 17–33% (Tarnopilska and Korotych, 2018).

Tovstukha *et al.* (2017) studied the growth of English oak stands aged 6–9 established with various stock types at 10 research plots in Ukrainian Left Bank Forest-Steppe (Sumy region). Oak stands were established on felling sites in fresh rich forest conditions. The study also showed that English oak stands established with container seedlings had a higher vitality (76–87%) compared with those established with bare-root seedlings (56–70%) or direct-seeded. Their crowns closed faster, and they moved to the next phase of stand development earlier. The stands established by sowing acorns had higher growth rate than that of the stands planted with bare-root and container seedlings. We obtained similar results in our study, but with a slightly higher vitality percentage.

In Ukrainian Right-Bank Forest-Steppe, starting from the age of 10, the average height of direct-seeded oak stands was 6–9% higher than that of the stands planted with seedlings (Ostapchuk *et al.,* 2018). The difference in the heights increased over time and varied from 11% to 19% at the age of 68. The productivity of oak stands established by sowing acorns was found to be higher by an average of 17%. The trees in oak stands established by sowing acorns self-prune better; accordingly, they have a better technical quality of wood and are also more resistant to forest diseases (Ostapchuk *et al.,* 2018).

Wilson *et al.* (2007) also indicated higher survival of container red oak seedlings compared to bare-root ones in Ontario (Canada) – 100% vs 75%. In addition, container seedlings reached a greater height during the first growing season. Container-grown seedlings were less stressed during transplanting to the forest area than bare-root ones. This was primarily due to the uninjured root system of seedlings in containers and their greater mass, which contributed to the intensive growth immediately after transplanting. Similar results were obtained by Woolery and Jacobs (2014) in the eastern United States for the growth intensity of containerized and bare-root red oak seedlings and their survival in the first year after transplanting.

The poor man-made oak reforestation by sowing acorns and planting container seedlings was emphasized by Schweitzer and Stanturf (1997) and Dey *et al.* (2006) in the Mississippi River Floodplain for pin oak (*Quercus palustris*

Muenchh.) and swamp white oak (*Quercus bicolor* Willd.). Only 9% of the area was successfully regenerated by these ways (Dey *et al.*, 2006).

The success of oak regeneration in such conditions can be partially improved by planting large seedlings, especially those with well-developed roots. Such criteria are met, in particular, by seedlings grown in containers (Schultz and Thompson, 1997; Kormanik *et al.*, 1998; Stanturf *et al.*, 1998; Gardiner *et al.*, 2002; Johnson *et al.*, 2002).

A comparison of survival rates of 3-year-old stands of *Quercus palustris* Muenchh. and *Quercus bicolor* Willd., established by container and bare-root seedlings, showed that container seedling survival for both oak species was 94% while for bare-root seedlings it was 76% for *Quercus palustris* Muenchh. and 54% for *Quercus bicolor* Willd. In addition, container seedlings of both oak species had higher biometric characteristics (height and root collar diameter) compared to bare-root seedlings (Dey *et al.*, 2006).

However, man-made reforestation using container seedlings is noted to be much more expensive than that with bare-root seedlings or direct seeding of acorns. Also, it requires a longer observation period before it can be recommended as a primary reforestation method in the Mississippi River Floodplain (Dey *et al.* 2006).

CONCLUSIONS

The article considers the growth dynamics (in height, diameter, current height increment and health condition) of oak stands within 11 years after their establishment by sowing acorns and planting bare-root and container-grown stock.

The highest values of height and diameter were recorded in 11-year-old oak stand established with container seedlings as compared with the direct-seeded stand and the stand established with bare-root seedlings.

The height increment intensity for the direct-seeded oak stand, calculated by an average current height increment, was higher than that for the stands established with container and bare-root seedlings.

The health condition index for the English oak stand established by sowing acorns was slightly higher for all the years of observations as compared with the stands established with container and bare-root seedlings.

The revealed features of oak tree growth in forest stands planted using various stock types indicate that direct oak sowing should be preferred for establishing artificial oak stands in fresh rich sites within forest-steppe zone, provided that a sufficient number of acorns is available and there is no threat of damage to acorns from rodents or wild boars. This method is inexpensive and best from biology point of view. Seeding provides high productivity and biological stability of future stands. Planting bare-root and containerized seedlings is also relevant in the absence of a sufficient number of germinative acorns for sowing.

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ECONOMICALLY SIGNIFICANT PRODUCTION OF *Secale cereale* L. AS FUNCTIONAL FOODS

SUMMARY

Secale cereale L. is second important bread grain, after wheat, and an economically important crop for functional food. Rye production parameters in world and in Serbia were analysed in this study and quality parameters. The average rye area (4.28 mill. ha) and production (12.12 mil.t) in world, in the five-year period, recorded a growth trend and average grain yield was 3.01 t ha^{-1}. In 2020 in the Republic of Serbia, the average grain yield of rye was 3,23 t ha^{-1}. Rye is considered a healthy cereal due to its high content of dietary fiber and is a rich source of vitamins B and E, minerals: Ca, Fe, F, P, K, Zn, Mn, Cu, K, which is why it is a suitable raw material for the production of functional food. Rye foods have beneficial effects on insulin, which may have positive implications for diabetes prevention. Development of innovative and tasty rye products is crucial in increasing awareness, consumption of rye foods and thus production.

Keywords: rye; production; yield; quality; functional food

INTRODUCTION

Rye (*Secale cereale* L.) is the most important bread grain, after wheat. Among cereals, rye has the highest content of dietary fiber and contains a broad spectrum of bioactive compounds (Koistinen *et al.*, 2018) that have been shown to affect physiological processes relevant to health. The growing

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demand for rye bread is due to the specific taste and aroma of bakery products. Diet is one of the most important environmental factors affecting the risk of developing non-communicable diseases and premature death (Mathers *et al*., 2008). High intake of whole grain cereals has consistently been associated with lowered risk of developing type 2 diabetes, cardiovascular disease, and some cancers (Aune *et al.*, 2016). Rye dietary fiber contain of arabinoxylan, cellulose, β-glucan, fructans, and lignin and are considered as the main driver of the health effects of rye foods. Of bioactive compounds in rye is phenolic acids, lignans, benzoxazinoids, and alkylresorcinols (Koistinen *et al*., 2017). In the list of phytochemicals detected in rye is found at almost 2000 chemical species (FooDB, 2018). Epidemiological studies have revealed that such food items are particularly protective against various diseases when consumed as part of the habitual diet (Schwingshackl *et al*., 2017).

Rye is a rich source of vitamins B and E, which is why it is a suitable raw material for the production of functional food. Rye is considered a healthy cereal due to its high content of dietary fiber (Rakha *et al*., 2010), it also contains minerals (Ca, Fe, F), lysine and oleic acid (Gumul *et al*., 2007). The main product is grain, which mostly used for bread and bakery product and in the industry for the production of alcoholic beverages. Grain of rye is utilised in a lot food products as well, in breakfast cereals, porridges, pasta, etc. Scientific research is focused on studying the possible health benefits and of using it for energy purposes. Rye is a real cereal perfectly adapted to different agroecological conditions, so that it has a very large area of distribution. It is mostly grown in harsher climates thanks to its resistance to frost and disease. Rye is major source of starch and energy. Rye grain contains nutritional components: protein, fat, vitamins (B vitamins), dietary fibre and phytochemicals. Improving drought tolerance has always been an important objective in many crop improvement (Psota *et al.,* 2009; Ikanović *et al.*, 2022). It is mostly grown in harsher climates thanks to its resistance to frost and disease. Rye bread stays fresh for a long time, is rich in vitamins of groups A, B and E and has high digestibility. Rye grain is smaller than wheat grain, which mostly depends on the variety and growing conditions. Grain is the raw material for obtaining starch and producing spirits. Rye sprouts are rich in vitamins, oils and mineral salts and are used in the food and pharmaceutical industry. Rye bread and bakery products play an increasingly important role in a healthy diet, it is recommended in the diet of diabetics and people with high blood pressure (Glamočlija *et al.*, 2015; Rajičić & Terzić, 2022). Because of the content of soluble dietary fiber, rye foods have cholesterol-lowering properties. Considering the many health benefits that rye food can have, its consumption is consumed.

The aim of this study was to determine the growth trend of rye production in the world and in our country and the possibility of using rye in functional food. Development of new tasty rye products, associated health claims, with application good marketing are crucial in order to increase

awareness and consumption of rye foods among consumers therefore, an increase in production.

MATERIAL AND METHODS

Rye production parameters in world and in Serbia were analysed in this study for the period 2016-2020. The research was based on available data from statistical publications (FAO, 2022). Rye quality parameters are also shown. The data (area, ha; yield, kg ha^{-1}; and production, t ha^{-1}) were analyzed by the descriptive statistical and mathematical procedures. The variability of the data was evaluated based on the value of the mean absolute deviations from the average and standard deviation - σ or SD. The seed production are presented by tables and figures.

RESULTS AND DISCUSSION

Rye production parameters. The average rye area (4.28 mill. ha) and production (12.12 mil.t) in world, in the five-year period, recorded a growth trend and average grain yield was 3.01 t ha^{-1}, Table 1. According to FAO data, the area under rye in the world, in 2020, in about 60 countries in the world was 4,446,927.00 ha, the total grain production was 15,022,273.00 tons and the average world yield was 3.38 t ha^{-1}. In the Republic of Serbia, the average yield of rye for the same year was 3,23 t ha^{-1}, Table 1.

Table 1. Average values of rye production in world and Serbia, 2016-2020

Parameter	Area, ha		Yield, t ha^{-1}		Production, t	
Year	**World**	**Serbia**	**World**	**Serbia**	**World**	**Serbia**
2020	4446927	4725	3.38	3.23	14022273	15240
2019	4249344	5046	3.02	2.57	12824590	12963
2018	4010321	4736	2.67	2.83	10716767	13418
2017	4299205	4673	3.03	2.41	13004048	11248
2016	4401000	4891	2.95	2.90	12999190	14200
Average	4281359	4814	3.01	2.79	12713374	10671
Std.dev.*	170684	153	0.25	0.32	1212251	5226
Source: FAO, 2022, *Authors calculation						

The largest production by countries, by top 10 in world, of rye in 2020 was in Rusia, Poland, Germany, Belarus, China, Canada, Spain, Ukraine, USA, Denmark and Argentina, Figures 1 - 2. The largest areas under rye in 2020 were in Russia 975.44 ha with a tendency to increase areas. In second place is Poland with 843.62 hectares. Germany, with 636,000 hectares, is the third in the world in sown areas and with an average grain yield above 5.5 t ha^{-1} (Rajičić &Terzić, 2022). The highest yields of rye in 2020 were achieved in Sweden at 6.20 t ha^{-1} and in Denmark at 6.07 t ha^{-1}.

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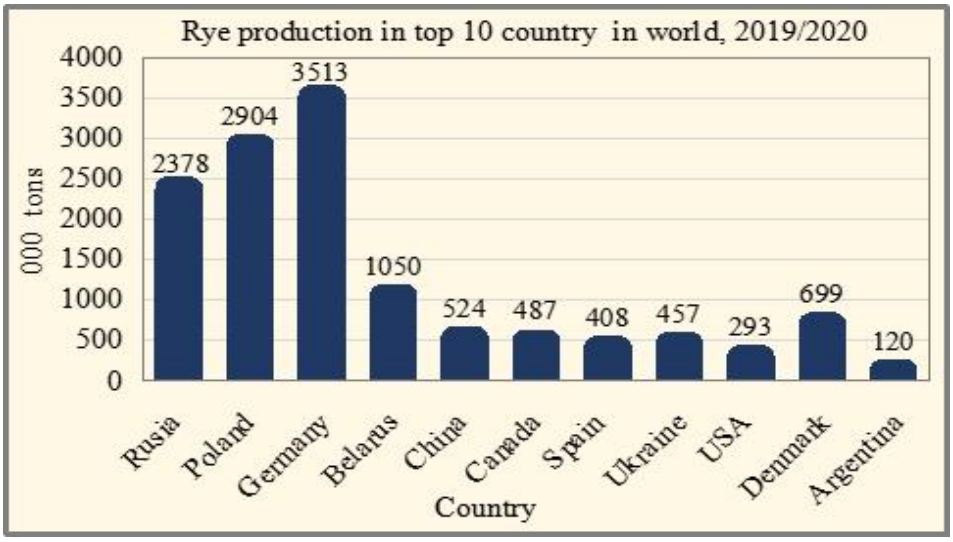

Figure 1. Average values of 10 top rye production in world, 2020

Rye is suitable for production according to the principles of organic agriculture because they have modest requirements for mineral substances, species and varieties are resistant or tolerant to diseases and pests, they have strong competitive or allelopathic properties in the fight against weeds (Ikanović & Popović, 2020). Oscillations in the temperature and the amount of precipitation during vegetation period rye are the main one factors of the yield instability. The drought has a main limiting factor of the plant production in Serbia and in the world. The introduction new of breeding programs for stress conditions will increase in the world. Areas have been increasing in recent years, both due to the interest of rye in organic production, production on acidic soils, and also due to the growing interest in biofuel production (Ikanović *et al*., 2022).

The best-known variety of rye in our country is NS Savo. NS Savo - is a mid-late, winter variety of rye, selected in the Institute of Field and Vegetable Crops, extremely stable and good yield, adaptable, high nutritional value and excellent resistance to low temperatures, to winter. Due to the longer spring period compared to other small grains, it is recommended to sow earlier, from September 20 to October 5. NS Savo has a plant height of 125-130 cm. Very good resistance to diseases and very good resistance to laying. Has an extremely stable and good yield, adaptable, high nutritional value and excellent resistance to

low temperatures, to winter. It is characterized by a weight of 1000 grains of 28-30 g, a hectoliter weight of 75-80 kg, an average protein content of 10-11%. The grain is rich in carbohydrates, plant fibers, minerals and vitamins (Rajičić & Terzić, 2022).

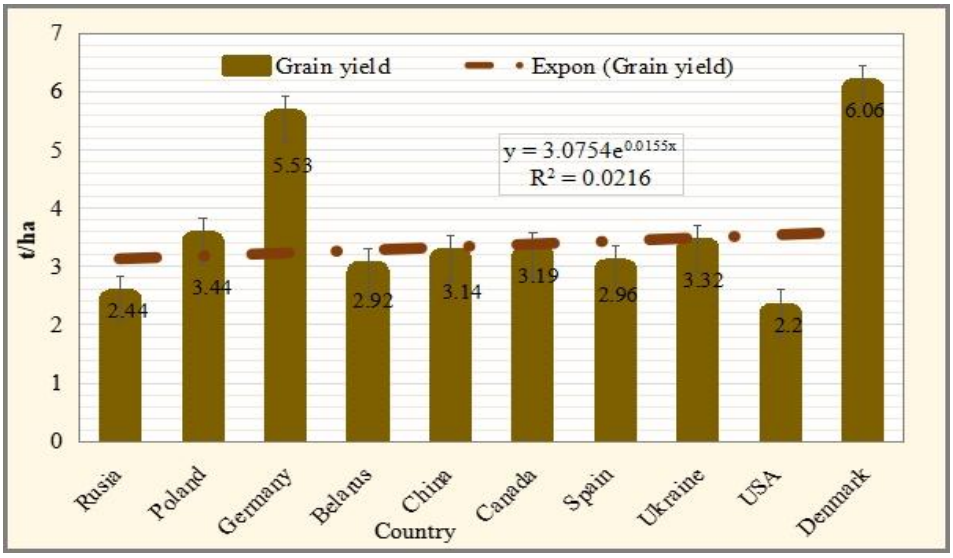

Figure 2. Average values of rye grain yield in 10 top in world, 2020

In Europe, wheat is grown on about 58 mil. ha, with an average yield of 3.72 t ha^{-1}, which gives total production of over 215 mil. tons. The biggest producers of wheat in the world are China, India, USA, Russia, France, while the largest European producers are: Russia, France, Germany, Ukraine and Great Britain. In Serbia, wheat is grown on an area of about 518 thousand ha, with with an average yield of 3.99 t ha^{-1} and with a total production of about 2.1 million tons, in Vojvodina grows wheat on an area of about 264 thousand ha, with an average yield of 4.50 t ha^{-1} and with a total production of about 1.19 mil. Tons (Aćin, 2016). Progress in breeding and cereals cultivation technology in the second half of the 20th century was very successful. Yield growth no longer follows the trend that had in the period from the 50^{s}-90^{s} of the last century and if the expected expansion of the human population continues, food shortages will become inevitable, and it is necessary that the growth of agricultural production at least follows the increase population (Khush, 1999). Climate change that stands out with rising temperatures, increasing evapotranspiration and decreasing precipitation, they will have significant negative effect on agricultural production (Popović *et al*., 2020a; 2020b, 2022). Dixon *et al.,* (2009), state that it will the number of people in the world by 2030 increase by about 40%. In order to meet the needs in that case for food, it would be necessary to stabilize agricultural areas at around 1.5 billion ha with a simultaneous annual increase in yield of 2%. Production based on respect for variety specificities is an untapped potential for

increasing the yield cereals, especially in the conditions of a changed climate (Janković *et al*., 2016; Aćin, 2016; Lakić *et al*., 2018; Filipović *et al*., 2020; Kolarić *et al*., 2021; Ljubičić *et al.*, 2021; Rakaščan *et al.*, 2021; Dražić *et al*., 2021). According to Glamočlija (2012), the influence of variety on height yield is about 40%, cultivation technology about 31-40%, and weather conditions of the year about 20-29%. It means that variety and cultivation technology are almost equal factors in achieving cereals yield. Only with respect demands of each genotype and by mitigating unfavorable weather phenomena through agrotechnical ones measure, conditions for high and stable production can be created. In the conditions of climate change the optimal sowing period can influence the reduction of the negative effects of unfavorable weather occurrence, in the sense of avoiding them in the critical stages of growth (Marinković *et al.*, 2010). One of the extremely negative phenomena in the predicted global warming will be frequent heat waves shocks that in the grain filling phase can have catastrophic consequences for yield and quality. Breeding, with better application of agrotechnical measures (first of all by adjusting sowing time), will play a major role in meeting the challenges of global change climate (Kobiljski & Denčić, 2001) in the evident conditions of global climate change.

Chemical composition of rye compared to wheat. Rye (*Secale cereale* L.) is the second most important crop after wheat for production of bread and other bakery products. The distribution of rye production differs from that of wheat, due to its demand for cooler growth temperatures and large differences in regional preferences for rye-based products (Mihhalevski *et al*., 2012; Poutanen *et al*., 2014; Wrigley & Bushuk, 2017). Rye is a rich source of vitamins B and E, which is why it is a suitable raw material for the production of functional food. Rye is considered a healthy cereal due to its high content of dietary fiber (Rakha *et al.,* 2010), it also contains minerals (Ca, Fe, F, P, K, Zn, Mn, Cu, K), lysine and oleic acid (Gumul *et al.*, 2007). Europe provides more than 85% of the world's rye production (12.8 million tons, 2019), including the leading rye producing countries: Germany, Poland, Russia, Denmark, and Belarus (FAO, 2022). Rye is important raw material for healthy safe foods. Rye chemical composition shows similarities with other cereals (e.g. wheat, barley, and triticale), however, it can be characterised by higher fibre (especially pentosan) (Mihhalevski *et al.,* 2012). Protein content of rye can vary in a wide range (8–15%) depending mainly on growth conditions. On the value of yield genotype has the higher influence ofcompared to environmental effect. The amino acid composition of rye proteins is better than that of wheat because there is a bigger one lysine content, but less in tryptophan and isoleucine. Starch dominates in rye and similarly to other cereals. The starch content of the rye grain varies 55-65%, in wheat 63–72%, and of barley, 50–64%. The lipid content of rye is similar to that of wheat (2–3%), also fatty acid composition shows similarities having linoleic acid (18.9–59.3%) as a major component. Mineral and vitamin composition of rye resembles to that of wheat (Table 2).

Table 2. Quality grain, mineral and vitamin contents of rye compared to wheat

Parameters	Rye	Wheat
Protein content (%)	10-14	13-17
Lipid content (%)	2-3	2-3
Starch content (%)	55-65	55-70
Faling number (s)	230-300	200-300
Minerals (mg)		
Phosphorus (P, mg)	1,806–4,220	1,500–5,400
Potassium (K, mg)	3,480–6,148	2,900–6,200
Calcium (Ca, mg)	157-1,447	370–1,220
Magnesium(Mg, mg)	920–1,602	900–2,900
Iron (Fe, mg)	27–129	28–42
Zinc (Zn, mg)	21–52	19–32
Manganese (Mn, mg)	20–75	5–49
Copper (Cu, mg)	3–13	4–7
Vitamins (mg kg^{-1})		
Thiamin (B1, mg)	4.0–4.6	5.0-12
Riboflavin (B2, mg)	1.8–1.9	1.0–3.1
Niacin (B3, mg)	12–15	41–64
Pantothenic acid (B5, mg)	10	7.7–9.1
Pyridoxine (B6, mg)	3.0–3.4	3.0–4.7
Folate (B9, mg)	0.48–0.52	0.35–0.56
α–Tocopherol (E, mg)	10–12	5–12

Source: Michela *et al.,* 1976; Frølich *et al.,* 2013; Sapirstein & Bushuk, 2016; Linina *et al.*, 2019; Bağcı *et al.*, 2019; Biel *et al*., 2020; Sakr *et al*., 2021;

The proportion of macronutrients (starch, lipids, proteins) in rye is the same as in other cereals. Unlike wheat, it contains a higher proportion of pentosan (about 10%), sugar and fiber, and a lower proportion of starch and protein, which affects the technological properties of rye flour. Rye proteins are low in gluten, which is why rye flour has weaker properties for the production of bakery products (Glamočlija *et al.,* 2015). Consuming rye grain products provides is the source of dietary fibre and bioactive compounds with potentially positive health implications. Cultivar, crop-year (weather conditions) and cultivar×crop-year interaction significantly ($P < 0.05$) affected rye grain protein content, starch content and Hagberg falling number. Protein content is in a significant negative correlation in relation tostarch content $r = -0.937$ (in population grain), $r = -0.944$ (cultivars grain), and protein content is in a medium strong negative correlation with falling number, $r = -0.549$ and $r = -0.573$ respective (Linina et al., 2019). The reduction in the amount of starch was accompanied by a significant change in the amylopectin/amylose ratio (Sakr *et al.*, 2021).

Rye and wheat represent the most important bread grains. Compared to wheat, rye contains less starch and protein, and more dietary fiber and free sugars.

Due to its increased fiber content and high vitamin and mineral content, rye can be successfully used in functional food formulations. Linoleic acid is the most abundant fatty acid in rye grain (55.6%). Rye contains it in slightly larger amounts compared to other cereals, followed by palmitic (16.5%), oleic (15.6%), linoleic (10.4%), eicosane (1.3%) and stearic acid (0.6%) (Arendt & Zannini, 2013).

Rye has higher iron, zinc, manganese, and copper contents, compared to other cereals. Rye is good source of α-tocopherol similarly to wheat, however, oat can be characterised by the highest vitamin E content (Mihhalevski *et al.*, 2012; Bağcı *et al.*, 2019; Frølich *et al.*, 2013; Sapirstein & Bushuk, 2016). Cereals are considered as good sources of phosphorus, potassium, and magnesium, as well as B vitamins (thiamin, riboflavin, niacin, pantothenic acid, and pyridoxine) and folate (Michela *et al.,* 1976).

Metabolic and healty effects of rye-based foods. Rye bread stays fresh for a long time, is rich in vitamins of groups A, B and E and has high digestibility. Rye grain is smaller than wheat grain, which mostly depends on the variety and growing conditions. Grain is the raw material for obtaining starch and producing spirits. Rye sprouts are rich in vitamins, oils and mineral salts and are used in the food and pharmaceutical industry. Rye bread and bakery products play an increasingly important role in a healthy diet, it is recommended in the diet of diabetics and people with high blood pressure (Glamočlija *et al.*, 2015; Rajičić & Terzić, 2022). Due to their extremely favorable nutritional value, rye is of great importance for health purposes. Many studies point to beneficial effects of rye-based foods on satiety, which is one plausible mechanism behind recently demonstrated beneficial effects on weight management. Also, they indicate beneficial effects of rye intake on inflammation and blood lipids. A challenge with rye-based foods is making them palatable and widely acceptable to consumers (Jonsson *et al.*, 2018).

Rye foods have beneficial effects on insulin metabolism compared with wheat bread under isocaloric conditions and at standardized amounts of available carbohydrates, which may have positive implications for diabetes prevention. An overview of potential health implications of rye consumption is provided in Figure 3, (Jonsson *et al.*, 2018).

Development of innovative and tasty rye products is crucial in increasing awareness, consumption of rye foods and thus production. The use in nutrition of whole grain foods may be beneficial in prevention of disease, for cardiovascular disease, type 2 diabetes, and colorectal cancer. Whole grain foods are one of the most relevant dietary factors for prevention of non-communicable diseases (Jonsson *et al.*, 2018). Rye breads, in comparison with wheat-based products, reduce the demand for insulin (Rosen *et al.*, 2009).

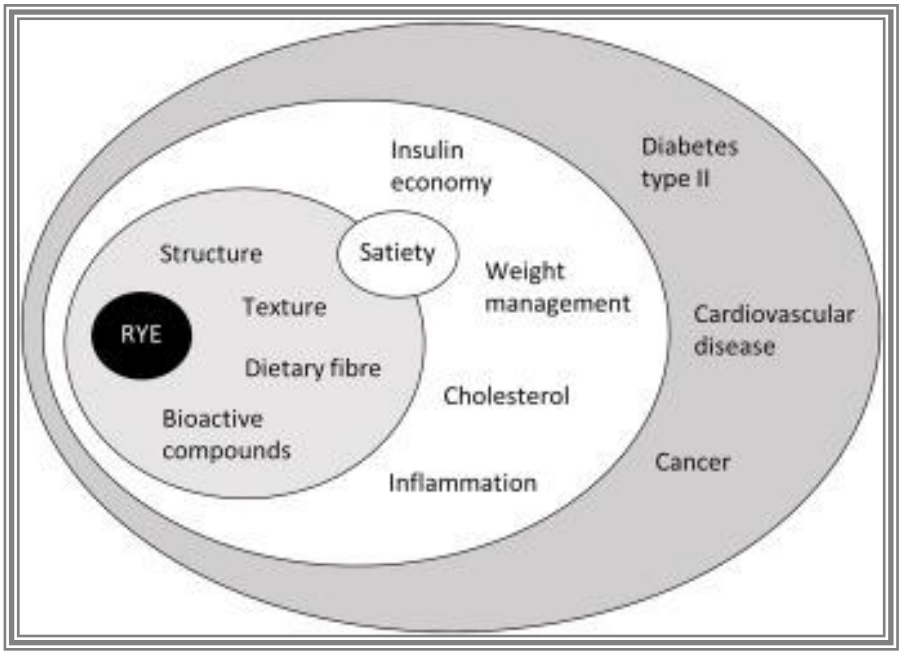

Figure 3. Overview of rye and potential health effects.

Rye is a cereal that is grown primarily for human consumption, and less often in the diet of domestic animals. Jovanović *et al*. (1984) point out that it is used in the diet of domestic animals smaller and narrow grain of rye, which, compared to whole grain, is richer in protein and cellulose, and poorer in starch. Rye can be used to feed all types of domestic animals, except for poultry, which is given less often. It should not be given in large quantities, and it is best to mix it with other cereals (Glamočlija *et al*., 2015; Rajičić & Terzić, 2022). Fresh above-ground biomass is used in mixtures with fodder peas, vetch or oilseed rape as voluminous fodder.

CONCLUSIONS

Small grains are of great economic importance in ensuring the food security of the population in the world and in our country. Grain and rye sprouts are rich in vitamins, oils and mineral salts, which is why the demand for rye in the world and production has increased. The area under rye in the world, in 2020, was 4,446,927.00 ha, the total grain production was 15,022,273.00 tons and the average world yield was 3.38 t ha^{-1} In the Republic of Serbia, the average yield of rye for the same year was 3.23 t ha^{-1}.

Due to their extremely favorable nutritional value, rye is of great importance in the food, pharmaceutical industry and for health purposes. Rye bread and bakery products play an increasingly important role in a healthy diet, it is recommended in the diet of diabetics and people with high blood pressure. Rye

play a very important role in providing complete functional nutrition for humans and domestic animals.

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FACTOR PRODUCTIVITY OF CHOSEN BULGARIAN AGRICULTURAL PRODUCTIONS

SUMMARY

The study focus on the Total Factor Productivity (TFP) for selected agricultural sectors (fruit growing, viticulture and dairy cattle breeding). The methodology used to determine TFP is according to Regulation (EU) 834/2014 laying down monitoring and evaluation framework for CAP 2014-2020. The Laspeyers index was applied to analyze the development of agricultural production and to explore the influence of different factors on the production output during the CAP implementation. The change was reviewed in comparison to a basis period and TFP was estimated as an index coefficient represented as industry output and the driving inputs (including labour, capital, land, variable production costs and subsidies).

The study reveals a significant change in TFP indexes over the period after Bulgaria's accession to the EU. The results show the TFP indexes decrease for fruits and cow's milk and increase for grapes (due to improved efficiency). There is a drop in production for grapes and cow's milk but a significant increase in production quantities for fruits. Meanwhile, the TFP index for fruit growing is slumping down, which is explicated by tangible enhancement in investment and capital costs during the covered period, which deems to improve the productivity and efficiency in the coming years. There is a need to increase and target the financial support for viticulture and dairy farming in order to hoist up the productivity and production outputs.

Keywords: Factor productivity, Fruit growing, Viticulture, Cow's milk, Input-output, Bulgaria

INTRODUCTION

In the years after Bulgaria's accession to the EU, the problems of Bulgarian agriculture continue to be related to productivity and overall production in the industry (IAE, 2020, Ivanov, 2020). Productivity per unit area or animal remains at levels lower than other European countries, with the exception of cereals and oilseeds (Ivanov *et al*., 2020). The main challenges

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facing Bulgarian agriculture are related with structural and sectorial imbalances, uneven distribution of financial support, polarization and overconcentration in the sector (Beluhova-Uzunova *et al*., 2018). Vegetable production, fruit growing, viticulture and animal husbandry are the sectors that experience the most serious difficulties for the development of their production and economic potential (Yovchevska, 2015). According to Manolova (2021), the more difficult access to financing for fruit growers complicates the widespread diffusion of innovative technological approaches, which is a challenge for the competitiveness of the sector. Another important problem, pointed out in the same study, is the lack of a long-term strategy for the development of fruit growing. Dimitrova and Dimitrov (2017) noted that in 2016, compared to 2007, the production of wine grapes in Bulgaria decreased by 41.6%, and of table grapes by 26.2%. The main reason for this is the drastic reduction in the area of vine plantations, which is influenced by the conditions of the economic environment in the branch - rising production costs, low purchase prices, insufficient financial support, a limited domestic market and weak competitive positions on the international market. Harizanova-Metodieva and Harizanova-Bartos (2019) stated the relatively uncompetitive production as a serious drawback of the Bulgarian dairy sector, which, in the conditions of strong international competition, leads to a decline in cow's milk production in Bulgaria after 2000.

Regardless of the different rates of development and importance of separate sectors for the national agriculture, similar problems are also observed in other European countries. According to Jandrić *et al*. (2015) challenges to the development of the dairy sector in Serbia, specifically in the Pešter region, are related to seasonal fluctuations in milk production, price variations, difficult terrain conditions, extensive gray market and the lack of funding and logistics. The authors stated that variable production costs – costs of materials, fuel and energy costs, costs of production services, and cost of sold goods create the greatest burden on operating income. In Romania (an EU member state), the development of the dairy sector faces a number of challenges, mainly related to the organization of farms in general, processing problems and high European milk quality standards (Chiurciu and Soare, 2019). Micu (2015) defined as an important problem for the development of the Romanian fruit growing sector the excessive fragmentation of the orchards and the small farm size. The author added to it the shortage of labor, as well as the lack of irrigation. Sudarić *et al*. (2020) considered that there are stimulating conditions for the sustainable development of the viticulture and winemaking in Croatia, but noted the high level of competition, relevant level of gray economy, high level of administrative legislation and a relatively small production capacity of manufacturers in comparison with other countries. According to Paschalidis *et al*. (2019) the existing problems in the wine sector in Greece stem mainly from high production costs and the accumulation of large stocks, as well as serious competition from countries outside the EU, especially from Latin America.

The level of production and productivity in agriculture are directly dependent on inputs, and the ratios between productivity and income on the one hand and production costs on the other determine the efficiency of the activity performed (Ivanov, 2021). The improvement of efficiency of resource used in vulnerable agricultural industries, such as the production of fruits, grapes and milk is important both for stabilizing the majority of family farms specialized in those sectors and to bring about the raising the value added from Bulgarian agriculture, which is seen as one of the most crucial issue.

The Total Factor Productivity (TFP) is a key economic concept characterizing the efficiency and competitiveness of agriculture, both at the national and at the sectoral levels (Buks, 2011, Čechura *et al*., 2014, Domanska *et al*., 2014). The complex matter of this concept allows its use as a tool for estimating the efficiency and optimization from the resource use and their return. The TFP is an index revealing the change in total output and the change in total inputs - labor, land, capital, specific costs of seeds, tillage, fertilizers and crop protection in crop production and feeding and veterinary expenses in dairy production (Čechura *et al*., 2014, Saikia, 2014, Ivanov, 2019, Kryszak *et al*, 2021). As a measure of the efficient use of factors of production, it makes possible to assess the dynamics of productivity over a period of time. According to Bacovic (2021), which was studied changes in the total factor productivity of the national economy and by different sectors, the application of the sectoral approach gives a more detailed picture of the overall structural changes in the economy. Following the author's logic, the changes in the factor productivity of selected agricultural productions for two comparable periods can outline the ongoing structural changes in agriculture.

The aim of the study was to determine the Total Factor Productivity (TFP) for selected Bulgarian agricultural sectors (fruit growing, viticulture and dairy cow husbandry) upon the period of Bulgaria's membership to the EU and to explore the influence and importance of different input and output elements for TFP evolution.

MATERIAL AND METHODS

The methodology used to carry out the TFP analysis was complied with general framework for monitoring and evaluation of the CAP 2014-2020, adopted by Regulation (EU) No. 834/2014 of the Commission. The contribution of the study is that it was done at the sectoral level and it was tried to differentiate thus to compare the factor productivity among key agricultural industries in Bulgaria.

The TFP indices are an integral part of the economic analysis and are widespread way for input-output research. The advantage of the approach is the relatively easiness in application and the adequate reliability of the results revealing the efficiency and interdependency between input factors and production output. The approach was considered inappropriate as a measure of technological change, as it suffers from conceptual and empirical shortcomings

(OECD, 2001). For agriculture, the data on the costs of different sectors and the input values were taken from the Farm Accountancy Data Network (FADN), whereas the output figures were adopted from Eurostat production statistics.

The main issue in carrying out the input-output analysis is the way by which those indicators will be normalized, weighted and aggregated. There are different approaches, like the main indices used being of Laspeyres, Paasche, Fisher, Tornqvist. Eltetö-Köves-Szulc, leading to a different presentation of the indicator (Latruffe, 2010). Each one of the indices implicitly predisposes a certain type of production function. For example, the Tornqvist index is compatible with a translog production function, while the Laspeyers index assumes a Leontief production function. (Capalbo *et al*., 1990).

For the purposes of the study, the analytical presentation of Laspeyers was chosen as the production factors used are available proxy indicators representing the key five input resources – land, capital, labour, materials and subsidies. As a measure of production efficiency, the TFP index makes it possible to assess the change in the use, efficiency and rationality of production factors in dynamics. The algorithm for estimations was represented as:

$$TFP_0^t\_L = \frac{O_0^t\_L}{I_0^t\_L} = \frac{\left(\frac{q_{1t}}{q_{10}} * w_{10} + \frac{q_{2t}}{q_{20}} * w_{20} + \ldots + \frac{q_{nt}}{q_{n0}} * w_{n0}\right) / (w_{10} + w_{20} + \ldots + w_{n0})}{\left(\frac{i_{1t}}{i_{10}} * x_{10} + \frac{i_{2t}}{i_{20}} * x_{20} + \ldots + \frac{i_{rt}}{i_{r0}} * x_{r0}\right) / (x_{10} + x_{20} + \ldots + x_{r0})},$$

where:

q_{it} – represents the quantity produced;

w_{it} – coefficient representing the share of the respective agricultural production in the total;

i_{1t}-cost elements;

x_{it}– coefficient representing the share of the element of total production costs.

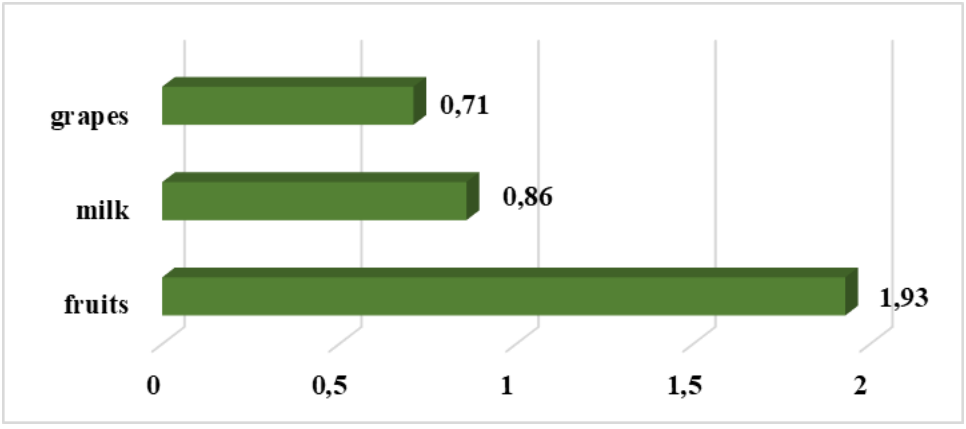
According to Ivanov (2019), the TFP is expected to display the common effects from comprised factors including the new technologies, efficiency changes, economy of scale, management capacity and organizational shifts, which may stand behind the TFP results. When calculating for one sector, the output indicator represented by production output was put in the equation numerator and it was yielded as ratio between production out in the new period to counterpart past time.

The basic period was 2008-2010, while the new, comparative period was the weighted average for 2016-2018. Thus, the development for the period during the Bulgaria's accession to the EU was analyzed.

RESULTS AND DISCUSSION

The Figure 1 shows the index of change in production output. A significant increase compared to the basic period was observed only in fruits, where the amount of production was almost doubled (the value of the production output index is 1.93). The reasons for this were attributed to the expansion of the area with orchards in the country, observed in recent years, and the increase in average yields.

Contrarily to the positive trend in fruit-growing, the drastic reduction of vineyards area, which was not compensated by the slow growth in average yields, led to a significant decline in grape production - from 285 thousand tons on average for the period 2008-2010 to 201.3 thousand tons averaged for the compared three years - 2016 to 2018 (-29.4%).



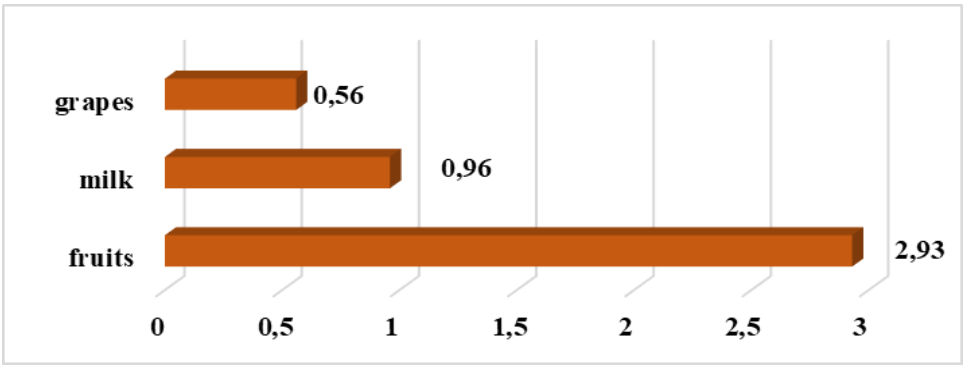
Sources: FADN, own calculations

Figure 1. Output index by sectors

The amount of cow's milk produced was also declining, albeit at a slower pace than in viticulture. The production decreased from 1081.2 thousand liters during the basic period to 933.8 thousand liters on average for 2016-2018 (-13.6%). The negative change in production was mainly due to the decrease in the number of dairy cows after Bulgaria entered into the EU, caused by unprofitable activities leading to bankruptcy and exit of sector from the small farm (Stoychev, 2021). Additionally, the increased requirements for the quality of milk, as well as the difficult access to subsidies contributed to this.

The change in the indices of inputs reveals different tendencies by sectors (Fig. 2). Resource intensity increased significantly in fruit growing, while in dairy cow husbandry and viticulture it declined. Such a development was due to the increase of production in fruit growing and from the strategy for optimization of the used resources in the production of grapes and cow's milk. The shrink of the activity in viticulture and dairy cow husbandry and the predominant farm goal to reduce the production costs, as safeguard behavior of producers against

the low purchase prices, determine the lower values of the input index in both sectors.



Sources: FADN, own calculations
Figure 2. Input`s index by sectors

Table 1 shows the change in the elements of input`s index for the three sectors observed. While intermediate consumption of fruit crops farms increased synchronously with the growth of production, in the cattle farms saw a decrease, which was more significant than the decrease in production and probably reflected a certain increase in herd productivity.

Table 1. Change in the elements of input`s index*

Sectors	**Elements of input`s index**				
	Intermediate consumption	Labour (AWU)	Capital	Utilized agricultural area (UAA)	Subsidies
Fruit growing	1.8	1.3	1.9	1.3	5.6
Viticulture	0.43	0.35	0.46	0.55	1.2
Dairy cow husbandry	0.77	0.41	1.5	0.65	1.4

Sources: FADN, Brussels; Own calculation
*Recalculated at sector level

The largest reduction in costs (for fertilizers, plant protection and other specific costs) was observed in viticulture (index of change of intermediate consumption for the both compared periods was 0.43). The reason for this was the price pressure on producers, as the purchase prices of wine grapes for the period 2008-2018 remained in the range of 500 - 650 BGN/t without VAT, according to the National Statistical Institute. Rising prices of inputs are pushing producers to reduce the amount of inputs in production in an effort to maintain the economic viability of their farms.

The provision of skilled labor is problematic not only for different sectors of agricultural production, but also for the entire agriculture. The results of a

questionnaire study, conducted by Bashev and Koteva (2022), show that a significant part of farms in the country have serious problems in effectively providing the necessary labor force. Popescu *et al*. (2021) indicated that Bulgaria is among the countries that remain below the EU average for agricultural output per AWU in 2020. The authors concluded that the efficiency of the use of labor force varies across EU member states depending on its efforts to increase net gross value and decrease labor input.

In the conditions of labor shortage, the reduction of labor input (AWU) is logical, as its replacement with automation and mechanization of production processes is increasingly sought. As can be seen from the data shown in Table 1, the most serious was the decrease in labor input in viticulture - the index of change was 0.35. The increasingly difficult provision of skilled workers and the growth of wages in agriculture is limiting the use of labor resources in the production of grapes. This has a negative impact on the production process in viticulture, both in the short and long term, due to non-compliance with the specific agricultural techniques of growing vines and hence obtaining unsatisfactory economic results. The labor resources used in dairy farming were also declining, while there was growth in fruit growing.

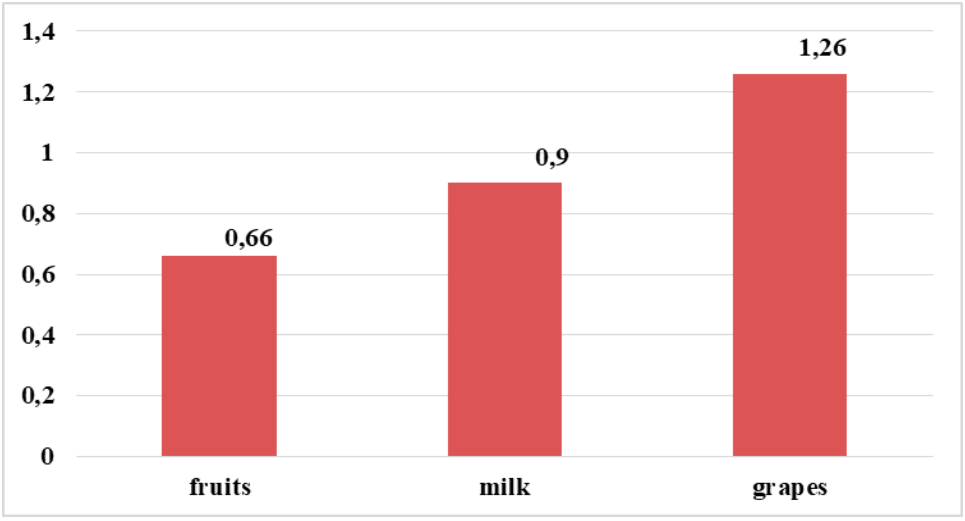
The shrinking scale of production activity in viticulture was the reason for the negative change in the capital used. The decrease was to a lesser extent than that of the labor resources, but it was indicative of the reduced level of technical security, which limited the possibilities for future increase of the efficiency of the production activity. Conversely, in the production of fruits and milk there was an increased use of capital on average for the three years 2016-2018 compared to the base period, which showed a desire to improve the technological level in the farms.

The index of change of agricultural area (UAA), as the main production factor in viticulture, clearly shows the significant contraction of production in the sector during the two periods compared. The area of harvested vineyards decreased by almost half - from 61,8 thousand hectares on average for the period 2008-2010 to 34 thousand hectares on average for 2016-2018 (-45%). In the opposite direction was the development of fruit growing, where there was an increase in area of orchards by 34%, which indicates the presence of entrepreneurial interest in the sector. The decrease in the land resources used in dairy cattle breeding was due to the decreasing number of dairy cows.

The data on financial support show that it was increasing in all sectors considered. This was mainly due to the general framework for amending the subsidies provided under the first pillar of the CAP. In cattle breeding, coupled payments increased significantly at the same time as decoupled direct payments. The index of change of subsidies had similar values in grapes and milk production, respectively 1.2 and 1.4. Significantly higher was the index value in fruit growing – 5.6, which is associated with the expansion of areas, respectively the positive development of activities in some fruit species, such as cherries, pears, raspberries, walnuts, apricots, plums and junipers and others fruits (Popov,

2021). In addition to directly supporting the income of agricultural producers, subsidies are also a factor that influences the price changes of some of the factors of production, such as the rent of agricultural land (Ivanov, 2021). According to Alexandri *et al*. (2020) subsidies do not contribute to productivity in the case of Romanian farms, with the exception of medium-sized dairy farms. A similar conclusion was reached by Martinho *et al*. (2022), according to which policy instruments under the 2013 CAP justify the use of more inputs (or at least at higher costs) for the same level of output, which negatively affects total factor productivity.

The results obtained are illustrated in Fig. 3 and it shows that an increase in factor productivity in the period 2008-2018 is observed only in the production of grapes.



Sources: Own calculations

Figure 3. Total factor productivity index by sectors

Given the previous analysis, the positive result is surprising and probably due to increased yields, but with a sharp reduction in inputs caused by the need for risk management and cost control. Grape production was shrinking, but at a slower pace than resource reduction. The high value of the TFP index – 1.26, indicates the efficient use of resources, which occurs mainly in agribusiness entities with a closed production cycle – growing vines, grape processing, wine production and sale. However, this direction of factor productivity development may be difficult to continue for a long period. The specifics of growing vineyards as permanent crops with a long period of operational life implies significant resource intensity, especially in terms of capital and specific production costs (for fertilizers, plant protection, etc.), as well as the need for skilled labor. Only in this way can be ensured stable economic results for years to come. Viticulture farms often are bound to the low-cost strategy, which leads

to an immediate increase in the resource efficiency, but if the production volume continues to decrease at a faster rate than the decrease in the physical volume of resources it is possible to expect a lower value of the index. Petrov and Borisov (2021) also noted the efforts of wine-growing enterprises to reduce production costs, which allows them to compete on price in the highly competitive conditions of the wine market in Bulgaria. This need arises from the relatively low incomes of consumers, for whom price is an important factor. At the same time, according to the authors, the reduction of production costs has a negative impact on the quality of production. Specifically for the wine market, branding based on the high quality of production allows flexibility in pricing, which is an important condition for overcoming competitive pressure and improving the market positions of wine producers (Nacka *et al*., 2019). It is explained to some extent by Dimitrov (2017), who points out that "as in the case of many other goods nowadays, it is easier to produce wine than to sell it" and the obstacles to sell wine grapes and wine at palatable price may undermine the viticulture output.

The increase of inputs in fruit growing was higher than that of production, which led to a decrease in factor productivity. According to Ivanov (2021), the main reasons for fluctuations in the quantities produced are largely due to the climatic conditions of the year. This raises the question of improving risk management mechanisms, both in farms (production insurance) and at the sectoral level (prevention of hail, frost, droughts, etc.). The participation of fruit growers in producer organizations can also support efforts in this direction. Dzhuvinov and Gandev (2015) considered that in Bulgaria the producers' organizations are insufficient, both by groups of fruit species and at the regional and national level, and they fail to protect the interests of their members. For comparison, in Poland, which is among the largest fruit producers in the EU, at September 28, 2018 there were 273 recognized fruit and vegetable producer organizations, associating 6.7 thousand members (Łakomiak and Zhichkin, 2020). According to Manolova (2021) to build highly competitive and efficient fruit growing sector in Bulgaria, producers, experts and institutions must join their efforts in the future.

The contraction of production was the main reason for the decline in factor productivity in dairy farming, but there were also regulatory factors, such as the introduction of European quality standards for raw milk, leading to a change in the specialization of some farms in the direction of beef production. According to Ivanov (2022), the low basic levels of productivity in the Bulgarian dairy sector compared to the leading countries in the EU, theoretically set prerequisites for improving its market competitiveness in the future period through appropriate interventions. The author indicated the critical situation of the middle class farms, which need for more investment and capitalization. Žáková Kroupova *et al*. (2020) cited research findings, according to which productivity growth in the dairy sector is mainly driven by technological change. Such an effect was observed in the Czech Republic, Estonia, Belgium, Hungary, Italy,

Sweden, Finland and Ireland. Following the example of European countries, it can be concluded that modernization and innovation are of key importance for increasing production, respectively factor productivity in the Bulgarian dairy sector.

The established values of the index of the total factor productivity are close to those, obtained by Ivanov (2019). The author pointed out that for the nine years of the period 2007-2017 an increase in productivity in the production of wine grapes was achieved – TFP index is 1.11 (+ 11%), while the productivity in cow's milk decreased (0.95). According to the cited study, Bulgarian agriculture follows a trend of optimizing inputs and production results, while in developed European countries the orientation is to maximize the result through high levels of resource intensity. In general, the factor productivity of agriculture in our country increased during the period 2007-2017, but continued to lag behind the average productivity in the EU (Ivanov, 2021). The implementation of innovations and better technological solutions can ensure the necessary growth rates of production and hence of productivity.

CONCLUSIONS

The results show that the TFP index decreases in fruits and cow's milk, but increases for viticulture. The increase in viticulture is due to a more significant reduction in inputs regardless the output is also down in the present years compared to the beginning of Bulgarian membership in EU. Generally, it testifies for an increase in production efficiency. However, such development in viticulture is thought to be difficultly maintained in the next years because the lack of space for replacing labor with capital.

The significant investment required to create new plantations of fruit trees and the lag in time to reach the full productivity in fruit crops are likely the cause for the downing value of TFP. Besides, the huge of the received subsides in the fruit growing farms by 5.6 times in the compared periods, which are liquid financial resource are considered as other reason for aggravated TFP index for fruit-growing sector. It is expected that in the coming years the value of the index in fruit production will increase with attaining full productivity of meantime founded plantations.

The importance of subsidies as an element of total factor productivity increased in the period 2008-2018. Despite the growth of financial support, the output is increase only in fruit growing. There is need to increase and focus financial support for viticulture and specialized dairy farms in order to maintain the current production volumes in the country.

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DETERMINATION OF MINERAL CONTENT IN CRANBERRIES (*VACCINIUM MICROCARPUM*) AND THEIR INFUSIONS CONSUMED IN KOSOVO

SUMMARY

After water, tea is the most widely consumed beverage in the world. In addition to its use as a beverage, tea is also known as a beverage that can have several health benefits, mainly due to the presence of nutrients. But, on the other hand, even very low concentrations of some metals can be toxic and can cause disorders and serious biological diseases. In this paper we have researched the content of micro and macro elements in chamomile tea samples of five different brands: sample 1 (Croatia), sample 2 (Kosovo), sample 3(Sri Lanka), sample 4 (Slovenia) and sample 5 (Kosovo) found in a local market in Pristina. Concentrations of the elements were determined by the method of atomic absorption spectroscopy (AAS). Microwave was used to decompose samples. Concentrations of metals in chamomile samples were: Na (1908-2480 mg/kg), K (3860-9294 mg/kg), Ca (11972-24269 mg/kg), Mg (1309-4287 mg/kg, Zn (10-33 mg/kg), Fe (86-302 mg/kg), Mn (69-217 mg/kg) and Ni (2-5 mg/kg) On the other hand metal concentrations in infusions of chamomile tea of these five brands at different times (3, 5 and 10 minutes) were: Na (1131-2233 mg/kg), K (3147-8999 mg kg), Ca (9189-22140 mg/kg), Mg (935-3899 mg/kg, Zn (2.1-29 mg/kg), Fe (6-47 mg/kg), Mn (37-177 mg/kg) and Ni (1-2 mg/kg). Of all the macronutrients (Na, Mg, K and Ca), calcium had the highest concentration which ranges from 11972-24269 mg/kg. By comparing the concentrations of all heavy metals (iron, zinc, nickel and manganese) iron content was the highest of all samples, in the range of 86 mg/kg - 302 mg/kg.

Keywords: *Vaccinium microcarpum*, cranberry, micro and macro elements, atomic absorption spectrometry.

INTRODUCTION

Tea is the most consumed beverage in the world after drinking water (Leyden *et al*., 2011). One of the main reasons for the popularity of tea is its

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positive impact on human health, mainly due to the presence of nutrients (Leyden *et al*., 2011; Petrović *et al*., 2015).

Tea is defined as a mixture of crushed plant parts intended for indoor or outdoor use. Important components of tea are flavonoids, tannins, catechins, caffeine, and thiamine, as well as theobromine, theophylline, and negligible amounts of carbohydrates, fats, and proteins. Of particular importance is the determination of minerals in tea which plays an important role in the quality of tea as well as in human health. Minerals are found in plants in form of ions, inorganic and organic salts. The content of mineral elements in plants can vary greatly. Changes in plant mineral content come from a number of factors, including plant species, plant age, soil pathological features, climate, and the implementation of agrotechnical measures. The human body needs adequate concentrations of these minerals to maintain normal function and maintain life. Deficiencies or excesses of minerals in the diet can lead to various health disorders (Perić-Grujić *et al.*, 2009; Mondal *et al*., 2004; Seenivasan *et al*., 2008; Fernandez-Caceres *et al*., 2001; Han *et al*., 2005; Pohl and Prusisz, 2007; Faiku *et al.*, 2019; Faiku *et al.*, 2018).

American cranberry (*Vaccinium macrocarpon* A.) and high bush blueberry (*Vaccinium corymbosum* L.) are perennial flowering plants from the family Ericaceae of the genus Vaccinium, commercial cranberry and blueberry varieties, in general, are indigenous to eastern and central North America including the eastern territories of Canada (Karlsons *et al*., 2018; Trehane, 2004). Commercial production of Vaccinium species in the United States of America has existed since the latter part of the eighteenth century (Eck, 1990).

Many species of *Vaccinium* have a long history of being used for medical purposes. Recent advances in nutrition science have shown that diet has a potential effect on human health and development, dietary guidance is persistent in recommending greater consumption of fruit and vegetables to promote health (Blumberg *et al*., 2016; Istek and Gurbuz, 2017).

There are numerous reports that the use of cranberry has a large number of benefits: affects the prevention of urinary tract infections, slows the spread of cancer and heart disease, lower blood cholesterol, and blood sugar levels, prevents dental disease and their gums, etc (Hwang et al., 2014; Koupy et al., 2015; Shi et al., 2017).

In different blueberries, the presence of essential elements is rarely reported as opposed to the presence of anthocyanins and phenolic compounds which is pretty much reported. (Pyrzynska, 2018). It should be noted, that many external factors as growth environment (soil, geographical conditions), cultivation and fertilization practices are widely diverse in different cranberry and blueberry production and wild harvesting countries and could contribute to the mineral composition of fruits.

The objective of this study is to establish the levels of some mineral and trace elements (Na, K, Ca, Mg Zn, Fe, Mn, and Ni) in these herbs and their infusions that are widely consumed in Kosovo.

MATERIAL AND METHODS

Apparatus and Reagents

The analysis of Na, K, Ca, Mg Zn, Fe, Mn, and Ni content were made with the M Series spectrophotometer type GE650416v1.26 Flame Mode Instrument. The device working parameters (air, acetylene, optics, and electronics) were adjusted for maximum absorption for each element. The standard solutions (1000 mg/L) were of analytical grade from Riedel de-Haen (Germany). The ultra-pure grade 65% nitric acid solution was used in the experiment (Merck, Germany). All solutions were prepared using deionized water.

The analyzed samples

The digestion of tea material was done with a microwave. For analysis the tea leaves samples of around 0.5 g were placed in Teflon digestion vessels; 7 mL HNO_3 65 % and 1 mL H_2O_2, 30% were added, and the vessels were capped closed, tightened, and laced in the rotor of the Analytik Jena microwave digestion. The digestion was carried out with the following programmer: step 1- temperature 180 °C, 10 min hold time with the power of 500 Wand and 45 bar pressure; step 2- temperature 180 °C, 15 min hold time, with the power of 500 W and 45 bar pressure. Finally, the vessels were cooled and carefully opened, and digests quantitatively transferred into 50 mL calibrated flasks.

The analyzed samples are commercial samples that were purchased in the markets Pristina - Kosovo in May 2021. Kosovo is situated in the Balkan Peninsula within the longitudes 41° 50′ 58″ and 43° 15′ 42″ and within the latitudes 20° 01′ 30″ and 21° 48′ 02″. Samples of *Vaccinium macrocarpon* teas were taken from different manufacturers (Sample 1 Croatia, sample 2 Kosovo, sample 3 Sri Lanka, sample 4 Slovenia, and sample 5 Kosovo). Infusions were prepared considering the recommended proportion for consumption: 1 bag (ca. 1-1.5 g) for a cup of 200 mL. Boiling water was added to the leaves and kept for 3, 5, and 10 min covered with a watch glass. A 250 μm polymeric membrane was used for filtration and, after cooling, beverages were acidified with distilled nitric acid to obtain a 0.2% (v/v) acid concentration. Quantitative analysis of all samples was performed on the M Series spectrophotometer type GE650416v1.26 Flame Mode Instrument. Experimental data were processed through the Anova software. For this purpose it was applied; Statistical analysis (descriptive statistics).

RESULTS AND DISCUSSION

Among all the metals tested, WHO classifies Ca, Na and Mg as minerals, Cr, Cu, Fe, Mn, and Zn as trace metals. Minerals or traces of metals, even the same ones, can have high importance and unwanted side effects at the same time for human health. The WHO recommends the Recommended Diet (RDA). RDA for Ca is 1.3 g, Na 1.5 g, Mg 0.42 g, Cr 35 μg, Cu 0.9 mg, Fe 18 mg, Mn 2.3 mg and Zn 11 mg. Table 1 presents data on samples of teas available in a supermarket in Pristina.

Table 1. Information on the herbal tea samples researched

Sample	Herb Latin name	Origin
1	*Vaccinium microcarpum*	Croatia
2	*Vaccinium microcarpum*	Kosovo
3	*V. microcarpum*	Sri Lanka
4	*Vaccinium microcarpum*	Slovenia
5	*Vaccinium microcarpum*	Kosovo

Table 2 shows the concentrations of Na, K, Ca, Mg, Zn, Fe, Mn, and Ni in the analyzed chamomile tea samples as well as the mean values, minimum, maximum and median values.

Table 2. Concentrations of metals in the analyzed microwave-decomposed *V. microcarpum* tea samples expressed as the dry sample mass.

	Concentration in mg/kg							
Elements	Na	K	Ca	Mg	Zn	Fe	Mn	Ni
Sample 1 (Croatia)	2289	3860	23175	1309	10	86	69	2
Sample 2 (Kosovo)	2216	8468	11972	2397	28	186	202	5
Sample 3 (Sri Lanka)	1962	6609	16320	2717	20	127	184	5
Sample 4 (Slovenia)	1908	8829	24269	2276	23	128	157	2
Sample 5 (Kosovo)	2480	9294	17598	4287	33	302	217	4
Mean	2171	7412	18666.8	2597.2	22.8	165.8	165.8	3.6
Minimum	1908	3860	11972	1309	10	86	69	2
Maximum	2480	9294	24269	4287	33	302	217	5
Median	2216	8468	17598	2397	23	128	184	4

Based on the results obtained, in microwave-decomposed samples of all macronutrients (sodium, magnesium, potassium, and calcium), calcium was most present followed by potassium, magnesium, and sodium.

Calcium is involved in a number of important physiological functions e.g., the rhythm maintenance of cardiac muscle and the excitability reduction both for nerves and muscles. Elevated Ca concentration (especially above 2.6 mM) is called hyperkalemia, which could be related to the development of myeloma, hyperparathyroidism, and vitamin D intoxication (Santulli and Marks, 2015). The average daily dose needed for calcium is 1000 mg. The presence of calcium in *V.*

microcarpum tea samples ranges from 11972 mg/kg in tea sample 2 (Kosovo) to 24269 mg/kg in tea sample 4 (Slovenia), figure 1(a) and table 2.

The amount of calcium (8900 mg/kg) found by Karlsons *et al*. (2018) in the leaf of *V. microcarpum* cultivated in Latvia is comparatively smaller than the amount of calcium found in *V. microcarpum* tea researched by us.

Potassium is very important because it is necessary for the maintenance of acid-base balance in the body, as well as osmotic pressure. It plays an important role in nerve impulse transmitting muscle contraction because it has the ability to increase the excitability of muscles and nerve cells. Potassium also has an effect on carbohydrate metabolism and membrane transport (Veljković and Vučković, 2010). The potassium content ranges from 3860 mg/kg in sample 1 (Croatia) to 9294 mg/kg in tea sample 5 (Kosovo), figure 1(a) and table 2. Potassium in teas *V. microcarpum* is in the amount of 7412 mg/kg which value is greater than the value found by Karlsons *et al*. (2018) 5400 mg/kg.

The recommended daily intake of potassium varies from 0.4 g for infants, 3.8 g for children aged 4-8 years, and up to 4.7 g for adolescents and adults.

Sodium, along with potassium, participates in the transfer of nerve impulses, affects membrane permeability, and maintains muscle tone. Lack of sodium in the body can cause a drop in blood pressure, which can lead to general weakness and loss of appetite (Veljković and Vučković, 2010). The sodium content in the tea samples consumed in Kosovo ranges from 1908 mg/kg to 2480 mg/kg, figure 1(a) and table 2. The recommended daily intake for infants is 0.12 g, for children aged 4-8 years, and 1.2 mg for adolescents, men, and women about 1.5 g.

Magnesium participates in many biochemical and physiological processes in the body. It is necessary for the normal functioning of many different enzymes (Shils *et al*., 1999). It is present in bones, tissues, organs, and blood. It is also essential for the normal functioning of muscles and the nervous system, supports immunity, makes bones strong, and promotes a normal heart rhythm. In addition, magnesium regulates blood sugar levels, affects blood pressure (Saris *et al*., 2000), and plays an important role in regulating blood pressure.

The magnesium content ranges from 1309 mg/kg in sample 1 (Croatia) to 4287 mg/kg in tea sample 5 (Kosovo), figure 1(a) and table 2. Karlsons *et al*. (2018) during the research of *V. microcarpum* leaf in Latvia have encountered the amount of 2200 mg/kg which compared to the amount of magnesium (2597.2 mg/kg) of the teas researched by us is a low value.

The high levels of potassium, magnesium, and sodium in all tested *V. microcarpum* tea samples are probably due to their high presence in the soil. It is well known that the main source of metals in food comes from the soil on which plant products grow. This brings about a clear link between the composition of the earth and the metals which enter the human body.

Iron is an essential nutrient for all life forms. Iron acts as a cofactor for many enzymes. It is essential for the transport of oxygen and the transfer of electrons. However, daily iron requirements can range between 8-18 mg for

humans. Because iron has pro-oxidant activity, it can be toxic in excessive concentrations (Wallace *et al*., 1992). The concentration of iron in *V. microcarpum* tea in samples 1, 2, 3, 4, and 5 consumed in Kosovo was 86 mg/kg – 302 mg/kg. Thus, iron content ranges from 86 mg/kg in sample 1 (Croatia) to 302 mg/kg in tea sample 5 (Kosovo), figure 1(b) and table 2.

The amount of iron (71.31 mg/kg) found by Karlsons *et al*. (2018) in a leaf of *V. microcarpum* cultivated in Latvia is comparatively smaller than the amount of iron found in *V. microcarpum* teas researched by us (165.8 mg/kg).

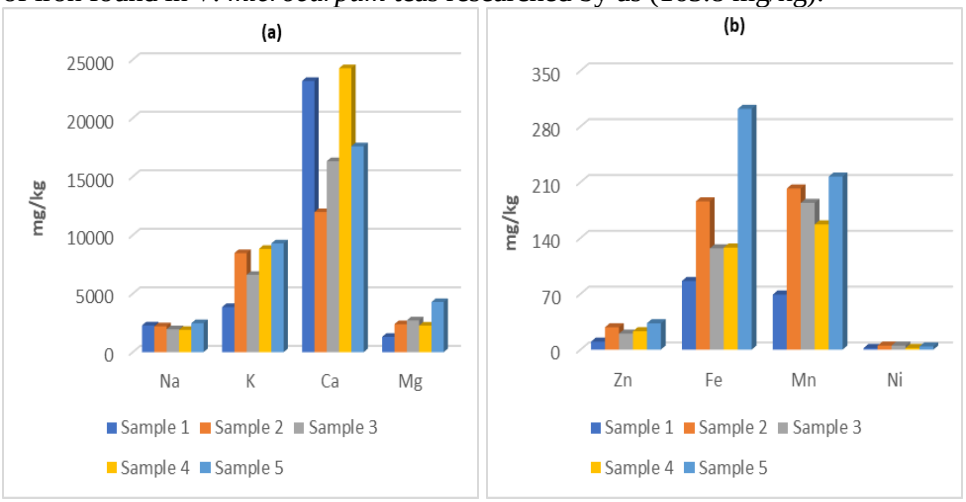

Figure 1. Concentrations of (a) Na, K, Ca, Mg and (b) Zn, Fe, Mn, Ni in mg/kg when we used the microwave to decompose samples

Manganese is a micronutrient found naturally in plants and animals (Powell *et al*., 1998). However, overexposure to Mn can have neurological effects which usually result from water consumption with very high levels of it (Powell *et al*., 1998). The manganese content in the samples of *V. microcarpum* tea consumed in Kosovo was in the range from 69 mg/ kg in sample 1 (Croatia) to 217 mg/kg in sample 5 (Kosovo). The amount of manganese (298 mg/kg) found by Karlsons *et al.* (2018) in a leaf of *V. microcarpum* cultivated in Latvia is comparatively bigger than the amount of manganese found in *V. microcarpum* teas researched by us (165.8 mg/kg).

The presence of small amounts of zinc is essential for increasing plant and animal life. Lack of zinc in the body causes growth retardation, anorexia, vomiting, etc. (Morgan, 1985). The amount of zinc in all samples was between 10 to 33 mg/kg, figure 1(b) and table 2. The zinc content in the samples of *V. microcarpum* tea consumed in Kosovo was in the range from 10 mg/kg in sample 1 (Croatia) to 33 mg/kg in sample 5. The amount of zinc found in our samples was approximately the same as the amount of zinc reported by Latvia scientists Karlsons *et al*. (2018). The amount of zinc reported by Karlsons *et al.* (2018) for *V. microcarpum* cultivated in Latvia in 2018 was 26.44 mg/kg, which according to our findings are approximate values.

Table 3. Concentrations of metals data (Na, K, Ca, Mg, Zn, Fe, Mn, and Ni; mg/kg) in cranberries tea infusions at different times.

Sample	Time/min	Na	K	Ca	Mg	Zn	Fe	Mn	Ni
1 Croatia	3	1131	3158	18455	952	2.5	44	37	ND
	5	2194	3469	13255	935	2.9	44	39	ND
	10	1384	3147	12691	979	2.1	47	41	1
2 Kosovo	3	1212	8098	9875	1150	21	23	156	ND
	5	1383	8368	9955	1143	19	25	159	2
	10	1613	8153	9189	1169	22	32	160	ND
3 Sri Lanka	3	1550	6132	10247	2176	5.0	16	135	ND
	5	1785	6129	10322	2184	5.8	16	139	1
	10	1704	5965	10311	2120	3.4	6	140	ND
4 Slovenia	3	1719	8659	21886	2045	19	15	122	ND
	5	1219	8721	22140	2153	21	26	125	ND
	10	1850	8716	22050	2145	17	19	127	ND
5 Kosovo	3	2029	8967	15468	3217	27	37	172	ND
	5	2233	8999	16422	3899	29	30	173	1
	10	2194	8442	15526	2681	21	32	177	1

ND: Not detected.

The concentration of nickel in all samples was lower than that of other metals and ranged from 2 mg/kg to 5mg/kg, figure 1(b) and table 2. Nickel is known to aid in pancreatic function and insulin production (Petrović *et al*., 2015). Table 3 shows the metal concentrations in *V. microcarpum* tea infusions at different times 3, 5, and 10 minutes, and table 4 presents descriptive statistics.

Table 4. Descriptive statistics of the concentration data (Na, K, Ca, Mg, Zn, Fe, Mn, and Ni; mg/kg) in cranberries tea infusions at different times samples.

Parameters	**Na**	**K**	**Ca**	**Mg**	**Zn**	**Fe**	**Mn**	**Ni**
Mean	1680	7008.2	14519.47	1929.87	14.51	27.47	126.8	1.2
Minimum	1131	3147	9189	935	2.1	6	37	1
Maximum	2233	8999	22140	3899	29	47	177	2
Median	1704	8153	13255	2120	19	26	139	1

The data in table 3, shows that the mineral content in the analyzed samples of *V. microcarpum* tea infusions is in a wide range.

The amount of sodium in samples of *V. microcarpum* tea infusions was as follows: sample1-Croatia (1131 mg/kg-2194 mg/kg); sample 2-Kosovo (1212 mg/kg-1613 mg/kg); sample 3-Sri Lanka (1550 mg/kg-1785 mg/kg); sample 4-Slovenia (1219 mg/kg-1850 mg/kg) and Sample 5-Kosovo (2029 mg/kg-2233

mg/kg). Sodium concentration ranges from 1131 mg/kg sample 1-Croatia, figure 2 (a) to 2233 mg/kg sample 5-Kosovo figure 6 (a). The average value of sodium concentration in the five samples analyzed was 1680 mg/kg. The amount of sodium was greater than the amount of manganese, iron, zinc, and nickel and was less than the amount of calcium, potassium, and magnesium.

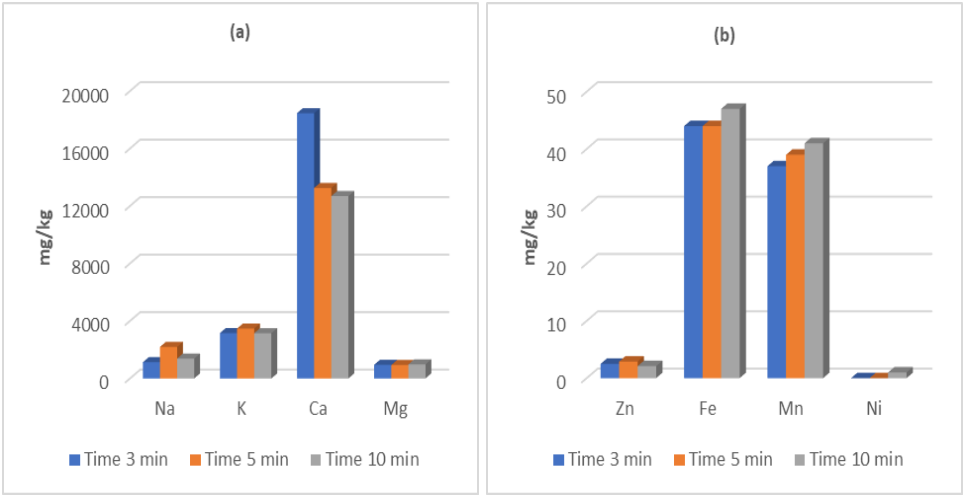

Figure 2. Concentrations of (a) Na, K, Ca, Mg and (b) Zn, Fe, Mn, Ni in mg/kg at different times in sample 1 (Croatia).

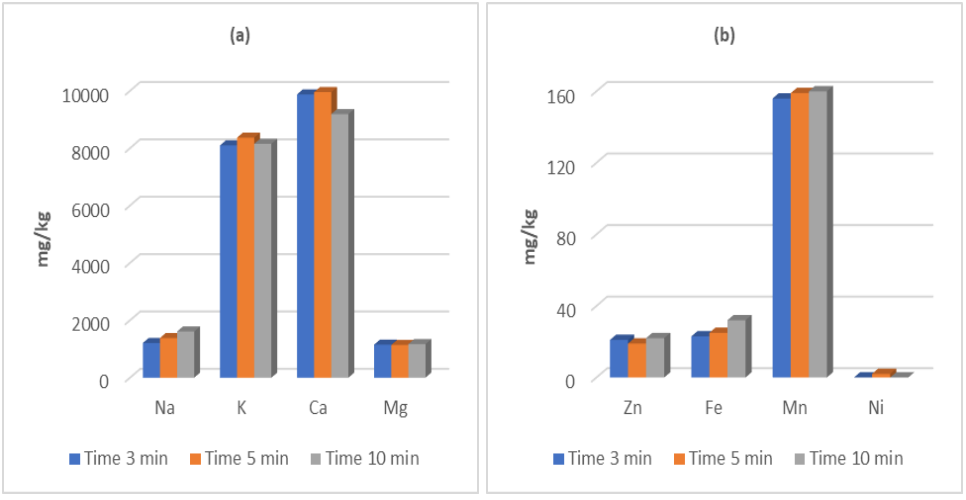

Figure 3. Concentrations of (a) Na, K, Ca, Mg and (b) Zn, Fe, Mn, Ni in mg/kg at different times in sample 2 (Kosovo).

The amount of potassium in samples of *V. microcarpum* tea infusions was as follows: sample 1-Croatia (3147 mg/kg-3469 mg/kg); sample 2-Kosovo (8098 mg/kg-8368 mg/kg); sample 3-Sri Lanka (5965 mg/kg-6132 mg/kg); sample 4-

Slovenia (8659 mg/kg-8721 mg/kg) and Sample 5-Kosovo (8442 mg/kg-8999 mg/kg). Potassium concentration ranges from 3147 mg/kg sample 1-Croatia (figure 2) to 8999 mg/kg sample 5-Kosovo, figure 6 (a). The mean value of potassium concentration in the five analyzed samples of tea infusions was 7008.2 mg/kg, table 5. The amount of potassium was greater than the amount of magnesium, sodium, manganese, iron, zinc, and nickel, and was less than the amount of calcium.

The amount of calcium in samples of *V. microcarpum* tea infusions was as follows: sample1-Croatia (12691 mg/kg-18455 mg/kg); sample 2-Kosovo (9189 mg/kg-9955 mg/kg); sample 3-Sri Lanka (10247 mg/kg-10322 mg/kg); sample 4-Slovenia (21886 mg/kg-22140 mg/kg) and Sample 5-Kosovo (15468 mg/kg-16422 mg/kg).

Figures 3 (a) and 5 (a), show that the amount of calcium ranges from 9189 mg/kg sample 2-Kosovo to 22140 mg/kg sample 4-Slovenia. The average value of calcium concentration in the five analyzed samples of *V. microcarpum* tea infusions consumed in Kosovo was 14519.47 mg/kg, table 5. So the amount of calcium was greater than the amount of all the other elements that were analyzed during our research.

The amount of magnesium in samples of *V. microcarpum* tea infusions was as follows: sample 1-Croatia (935 mg/kg-979 mg/kg); sample 2-Kosovo (1143 mg/kg-1169 mg/kg); sample 3-Sri Lanka (2120 mg/kg-2184 mg/kg); sample 4-Slovenia (2045 mg/kg-2153 mg/kg) and Sample 5-Kosovo (2681 mg/kg-3217 mg/kg).

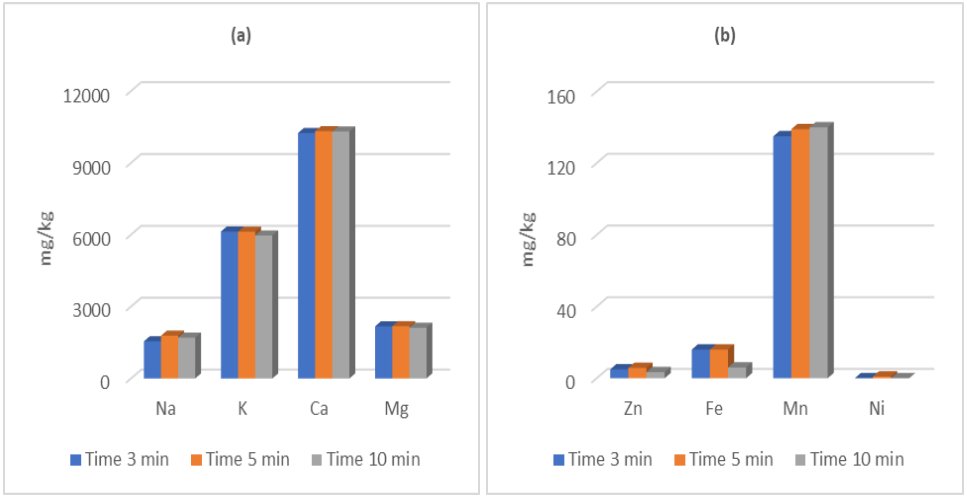

Figure 4. Concentrations of (a) Na, K, Ca, Mg and (b) Zn, Fe, Mn, Ni in mg/kg at different times in sample 3 (Sri Lanka).

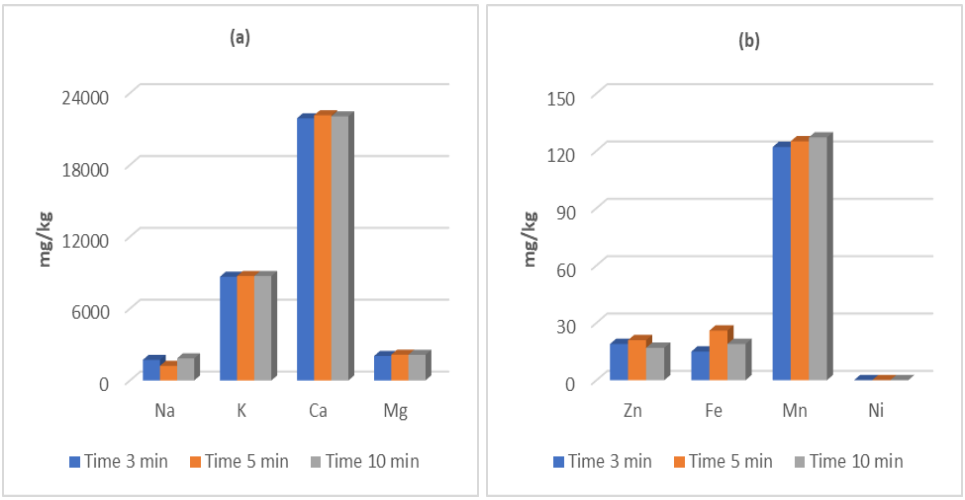

Figure 5. Concentrations of (a) Na, K, Ca, Mg and (b) Zn, Fe, Mn, Ni in mg/kg at different times in sample 4 (Slovenia).

The magnesium concentration ranges from 935 mg/kg sample 1-Croatia (figure 2) to 3899 mg/kg sample 5-Kosovo, figure 6 (a). The average value of magnesium concentration in the five samples analyzed was 1929.87 mg/kg, table 5. So, the amount of magnesium was greater than the amount of sodium, manganese, iron, zinc, and nickel and was less than the amount of calcium and potassium.

The amount of zinc in samples of *V. microcarpum* tea infusions was as follows: sample1-Croatia (2.1 mg/kg-2.9 mg/kg); sample 2-Kosovo (19 mg/kg-22 mg/kg); sample 3-Sri Lanka (3.4 mg/kg-5.8 mg/kg); sample 4-Slovenia (17 mg/kg-21 mg/kg) and Sample 5-Kosovo (21 mg/kg-29 mg/kg). Also from figures 2 (b) and 6 (b), it can be seen that the amount of zinc ranges from 2.1 mg/kg sample 1-Croatia to 29 mg/kg sample 5-Kosovo. The average value of zinc concentration in the five analyzed samples of *V. microcarpum* tea infusions consumed in Kosovo was 14.51 mg/kg, table 5. So the amount of zinc was greater than the amount of nickel and was much smaller than the amount of other elements that were analyzed in *V. microcarpum*tea infusions during our research.

The amount of iron in samples of *V. microcarpum* tea infusions was as follows: sample1-Croatia (44 mg/kg-47 mg/kg); sample 2-Kosovo (23 mg/kg-32 mg/kg); sample 3-Sri Lanka (6 mg/kg-16 mg/kg); sample 4-Slovenia (15 mg/kg-26 mg/kg) and Sample 5-Kosovo (30 mg/kg-37 mg/kg). Also from figures 2 (b) and 6 (4), it can be seen that the amount of iron in *V. microcarpum* tea infusions ranges from 6 mg/kg sample 3-Sri Lanka to 47 mg/kg sample 1-Croatia. The average value of iron concentration in the five analyzed samples of *V. microcarpum* tea infusions consumed in Kosovo was 27.47 mg/kg, table 5, while from Petrović reports, the level of iron concentration in chamomile teas (*Matricaria chamomilla* L.) was 1.9 to 7.4 mg/kg, (Petrović et al., 2015), which

has values much lower than our results and I think this may be as a result of research into different teas.

So the amount of iron was greater than the amount of zinc and nickel and was much smaller than the amount of Ca, K, Mg, Na, and Mn which were analyzed in *V. microcarpum* tea infusions during our research.

The amount of manganese in samples of *V. microcarpum* tea infusions was as follows: sample1-Croatia (37 mg/kg-41 mg/kg); sample 2-Kosovo (156 mg/kg-160 mg/kg); sample 3-Sri Lanka (135 mg/kg-140 mg/kg); sample 4-Slovenia (122 mg/kg-127 mg/kg) and Sample 5-Kosovo (172 mg/kg-177 mg/kg). The manganese concentration ranges from 37 mg/kg sample 1-Croatia, figure 2 (a) to 177 mg/kg sample 5-Kosovo, figure 6 (a). The mean value of manganese concentration in the five analyzed samples of *V. microcarpum* tea was 126.8 mg/kg, table 5. Krstić reported that their actions on Mn in different tear samples ranged from 1.82-651.04 mg/kg (Krstić *et al*., 2021). Also, Podwika and Kleszc reported high Mn levels in teas of different types and different colleagues also reported high Mn ranging from 457 to 2210 mg/kg (Podwika *et al*., 2018), hence our comparable results, table 5.

In most of the samples analyzed nickel was below the detection limit. Figures 2 (b), 3 (b), 4 (b), and 6 (b) show that the amount of nickel ranges from 1 mg/kg (sample 1-Croatia, sample 3-Sri Lanka and sample 5-Kosovo) to 2 mg/kg (sample 2-Kosovo).

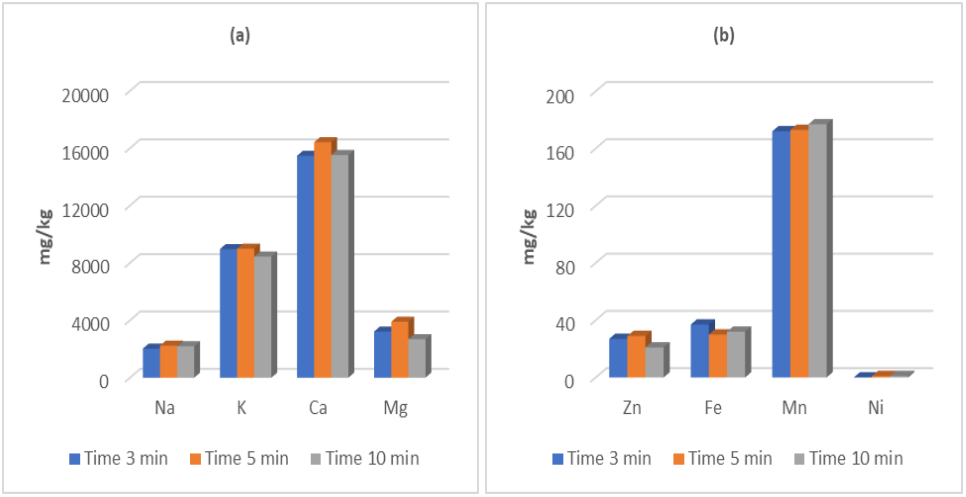

Figure 6. Concentration of (a) Na, K, Ca, Mg and (b) Zn, Fe, Mn, Ni in mg/kg at different times in sample 5 (Kosovo).

The average value of nickel concentration in the five analyzed samples of *V. microcarpum* tea infusions consumed in Kosovo was 1.2 mg/kg, and it is comparable to the values reported by Krstić for the Sambucusnigra L. tea type, where the concentration level is 0.97 mg/kg (Krstić, 2021).

CONCLUSIONS

In this research, we have analyzed the content of micro and macro elements in *V. microcarpum* tea samples found in a local market in Pristina. Metal concentrations were determined by the method of atomic absorption spectroscopy. So, the objective of this study was to determine the levels of some minerals and trace elements (Na, K, Ca, Mg, Zn, Fe, Mn, and Ni) of the five trademarks which are widely consumed in Kosovo.

Based on the results obtained in this paper, the presence of micro and macro elements in *V. microcarpum* tea samples were within the permissible limits, including the presence of heavy metals. All samples of *V. microcarpum* tea which were tested showed high concentrations of macro elements that can meet the daily needs of these nutrients.

Finally, we suggest that there is a need for more rigorous and consistent controls of the herbal products available in our markets.

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PHENOTYPIC CHARACTERISTICS OF GATACKO CATTLE FROM THE REGION OF HERZEGOVINA

SUMMARY

For the purpose of phenotypic characterization of Gatacko cattle, measurements were performed on a total of 92 individuals of different age categories, at the beginning of 2022 in the Gacko municipality, at 15 locations (Bosnia and Herzegovina, Republic of Srpska). Phenotypic measures (parameters), 15 in total, are divided into two groups, for the sake of clarity, as follows: a) height at withers (WH), back height (BH), loin height (LH), body length (BL), chest width (CW), chest depth (CD), chest girth (CG), hip width (HW), pin bones width (PBW), front shin girth (FSG), back shin girth (BSG); b) head length (HL), forehead width (FW), horn length (HNL), horn girth (HNG). For the purpose of data analysis, the aim was to determine the variability of the average values of the examined parameters, body indices, correlation of investigated parameters and significance of calculated correlation coefficients. Based on the results of our research on Gatacko cattle in the Gacko municipality, Republic of Srpska, Bosnia and Herzegovina (B&H), an increase in most of their body dimensions is noticeable.

Keywords: Gatacko cattle, genotypic traits, body index, phenotypic correlation

INTRODUCTION

Food production is one of the strategic priorities in the future. Commercial breeds of cattle, which dominate in industrial production, cannot fully meet the expectations and demands of consumers. There are several reasons that confirm the above, and the most important one is the reduced

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resistance and productivity of commercial breeds in conditions where domestic (autochthonous) breeds give their maximum, therefore Gatacko cattle.

However, the displacement of rural areas, the neglect of livestock production, especially in places (areas) where commercial cattle breeds could not be used (due to poor conditions), has led to a reduction in the number of domestic cattle breeds. Consumers have long wanted to have autochthonous products in their menu, and the sharp increase in demand for domestic products has influenced the changes that are taking place and which, among other things, contribute to work on the conservation of genetic resources. In the Republic of Srpska and B&H, there has been a general decrease in the cattle population, and thus the autochthonous breed that is the subject of this paper – Gatacko cattle (*Law on livestock, Official Gazette of the RS, 44/15, Article 40, paragraph 2*). The Gatacko cattle was created by breeding Busa with Viptal and Oberintal cattle. Based on the phenotype, this breed belongs to the group of short-legged cattle. It is short-headed with a wide and uneven forehead. It has a dark pigmented muzzle with a light edge. The horns are thin, pointed forward. The color of the hair is grey, it can also be brown, with dark shading on certain parts of the body. Females weigh about 400 kg and males about 750 kg. Gatacko cattle is most valued for its milk production, which goes up to 2,500 litres (Katica *et al*., 2004).

The contribution of these researches lies in the continuation of previous research (*Nikitovic et al*., 2021), on morphometric parameters of autochthonous cattle breed (Gatacko cattle), which provides further material for research towards genotyping of this autochthonous cattle breed and establishing the breeding goals.

Morphometric research is very important because it is the first step when it comes to more complex analysis such as molecular researches. Morphometric measures are used for evaluation of individual necks and it gives as a clear picture of population uniformity.

The aim of this research was to analyze phenotypic (morphometric) parameters (15 in total), separated into two logical groups of parameters by region (11 + 4), a total of 92 necks/individuals of autochthonous breed Gatacko cattle (Gacko municipality, B&H – Republic of Srpska). For the purpose of data analysis, the aim was to determine the variability of average values of the examined parameters, body indices, correlation of the investigated parameters and to determine the significance of the calculated correlation coefficients.

MATERIAL AND METHODS

Experimental measurements were performed at the beginning of 2022, in the Gacko municipality (Bosnia and Herzegovina, Republic of Srpska), at 15 locations (villages). For the purpose of morphometric (phenotypic) characterization of Gatacko cattle, measurements were performed on a total of 92

(in words: ninety-two) individuals of different age categories. There was no emphasis on gender differences in this research/paper. Based on the registry (ear tag), and the statement of the owners, the age of each cow was defined. Phenotypic measures (parameters), 15 in total, are divided into two groups, for the sake of clarity, as follows:

a) height at withers (WH), back height (BH), loin height (LH), body length (BL), chest width (CW), chest depth (CD), chest girth (CG), hip width (HW), pin bones width (PBW), front shin girth (FSG), back shin girth (BSG);

b) head length (HL), forehead width (FW), horn length (HNL), horn girth (HNG)

Measuring of body dimensions (morphometric, phenotypic) were performed using zootechnical aids. Zootechnical aids that were needed to measure this group of parameters are the cattle tape and Lydtin's rod (*Lalovic* and Z*dralic*, 2018). The rule states that body measurements (morphometric) must be performed in the way where the animals/necks must stand on a flat and firm surface. During the measurement, it is best that the animal stands in a natural position, i.e., that it rests evenly on all four legs. Also, as a rule, measures are taken first from the left side, then from the right side of the animal, after which the average value for the measured parameter is calculated (*Krajinovic et al*., 2000). Lydtin's rod measured linear body dimensions: height, length, depth and width, while the following body dimensions were measured with a cattle tape: head length, forehead width, horn length, horn girth, chest girth and shin.

The measures determined by the above-mentioned aids are expressed in absolute numbers, expressed in units of measure and serve to assess the development of the individual. For the sake of clearer picture, relative indicators of physical development, i.e., body indices, were also calculated. The body indices represent the ratio of one measured body parameter to another, in this case to the height at withers (*Nikolic* and *Simovic*, 1985). Indices calculated in relation to the withers height are mentioned in Tables 1 and 2 (chapter Results and discussion). In addition to them, the following indices were calculated: format index (obtained by dividing the average body length by the average height at withers, expressed as a percentage), chest index (obtained by dividing the average chest width by the average chest depth, expressed as a percentage), density index (obtained by dividing the average chest girth by the average body length, expressed as a percentage), massiveness index (obtained by dividing the average chest girth by the average height at withers, expressed as a percentage). The indices are shown in Table 3 (Results and Discussion chapter). The reason of showing these values also lies in the information about the extra- and intrauterine development of the individual, since body indices direct us to assess the general type of animal constitution, thus completing the picture of the individual's exterior.

In order to process the collected data for each parameter, descriptive analysis (statistical) was determined using Microsoft Office Excel 2010, while the

R Core Team package (2015) was used to calculate the phenotypic correlation between all morphometric parameters.

RESULTS AND DISCUSSION

Phenotypic (morphometric) measurements/characterization of the autochthonous breed Gatacko cattle were performed on a total of 15 (fifteen) body parameters, of which the first 11 (eleven) are described in Table 1 and the other 4 (four) in Table 2, since these measures are being performed on the head of the animal, for the sake of clarity, the results are highlighted in Table 2.

The average values and variability of 11 (eleven) body parameters of Gatacko cattle are shown in Table 1.

Table 1. Average variability of body parameters in Gatacko cattle

Characteristic	$\bar{X}$	$S_{\bar{x}}$	Sd	CV (%)	I*	Min.	Max.
Hight at withers (cm)	133.3	0.42	4.068	3.05	1.00	121	154
Back height (cm)	131.7	0.43	4.174	3.17	0.98	120	154
Loin height (cm)	133.1	0.42	3.989	2.99	0.99	121	154
Body length (cm)	154.8	0.60	5.791	3.74	1.16	131	172
Chest width (cm)	59.3	0.65	6.197	10.44	0.45	44	68
Chest depth (cm)	75.5	0.70	6.764	8.96	0.57	47	84
Chest girth (cm)	183.4	0.86	8.275	4.51	1.38	167	232
Hip width (cm)	61.4	0.31	2.956	4.79	0.46	49	72
Pin bones width (cm)	43.6	0.45	4.304	9.87	0.33	25	53
Front shin girth (cm)	14.8	0.19	1.784	12.07	0.11	11	19
Back shin girth (cm)	14.4	0.18	1.765	12.28	0.10	11	18

* Indices in relation to the height at withers

The determined height at withers in this research (133.3 ± 0.42 cm), is close to the values determined in the research by *Nikitovic et al*. (2021), which amount to 133.3 cm (with the proviso that this research worked on defining body measurements on 288 necks). The variation width for this parameter ranges from 121 to 154 cm (33 cm). It is interesting to add, according to research by *Ilancic* (1952), when it comes to the height at withers in this breed, the value was on average 112.56 cm, which significantly exceeds the value of height at withers obtained in this research. It also leads us to the conclusion that the format of the Gatacko cattle has been changed (upgraded), due to numerous paragenetic factors (better nutrition, better keeping conditions). The average back height was 131.7 ± 0.43 cm, with a variation width of 120 to 154 cm, which is in accordance to the results by *Nikitovic et al.* (2021). The average value of the loin height in the research was 133.1 ± 0.42 cm, while in the research by *Varatanovic* (2018) the obtained values differ and range from 128.83 to 134.00 cm. When it comes to the body length parameter, the average value in this research was 154.8 ± 0.60 cm, which is in accordance to the research by *Nikitovic et al.* (2021), where the average value was 156.4 cm. In the research by *Gutic et al.* (2003), this value was slightly higher (159.30 cm), while it was lower in the research by *Varatanovic*

(2018) where the value amounted to 142.56 cm. Based on the measures determined in the chest region (width, depth, girth), the average chest width was 59.3 ± 0.65 cm, the average chest depth was 75.5 ± 0.70 cm and the average value for the chest girth was 183.4 ± 0.86 cm. In the research by *Nikitovic et al.* (2021) the obtained average values were slightly higher when it comes to chest width and chest depth (64.3 cm and 78.5 cm), while they were in accordance when it comes to chest girth (183,6 cm). Also, in the research by *Varatanovic* (2018), a consistent average value for chest girth in Gatacko cattle was recorded. Far lower values for chest girth can be found in the research by *Pajanovic* (1961), values averaging 151.41 cm, 147.25 cm and 148.07 cm (at three different locations), as well as in the research by *Popovic et al.* (1979) where the average value of chest girth was 166.21 cm. The average value for the hip width in the research was 61.4 ± 0.31 cm, ranging from the minimum to the maximum value of 49 to 72 cm. The calculated values are in accordance to the results obtained in the research by *Nikitovic et al.* (2021), while it is significantly higher than in the research by *Gutic et al.* (2003), by 9.36 cm. The pin bones width in the research was 43.6 ± 0.45 cm. When it comes to the average values of the shin girth, both front (14.8 ± 0.19 cm) and back (14.4 ± 0.18 cm), the obtained values were slightly higher than the values (13.7 cm and 13.1 cm) obtained in the research by *Nikitovic et al.* (2021). It is interesting to note that in the research by *Gutic et al.* (2003), the shin girth averaged 20.24 cm, so the calculated value is much higher compared to our research, with the emphasis on the coefficient of variation (CV). The coefficient of variation of the front and back shin girth was 12.07% and 12.28%, while the values of these two parameters in the research by *Nikitovic et al.* (2021) was 7.23% and 7.63%. In the research by *Gutic et al.* (2003) the calculated value was 4,45%. High coefficients of variation indicate the "instability" of the obtained result due to certain disturbances caused during the measurement (usually restless animals).

The average values and variability of 4 (four) parameters measured on the head of an animal (Gatacko govedo) are shown in Table 2.

Table 2. Average variability of body parameters (head measurements) in Gatacko cattle

Characteristic	$\bar{X}$	$S_{\bar{x}}$	Sd	CV (%)	I*	Min.	Max.
Head length (cm)	47.4	0.24	2.277	4.80	0.36	42	53
Forehead width (cm)	25.0	0.22	2.130	8.52	0.19	16	29
Horn length (cm)	14.7	0.40	3.804	25.55	0.11	7	26
Horn girth (cm)	13.0	0.20	1.955	14.99	0.09	9	18

* Indices in relation to the height at withers

The average values of phenotypic parameters, measured on the head of animals, for the head length were 47.4 ± 0.24 cm, for the forehead width 25.0 ± 0.22 cm, for the horn length 14.7 ± 0, 40 cm and for horn girth 13.0 ± 0.20 cm. The obtained results were in accordance to the results by *Nikitovic et al.* (2021), with the exception of horn length (13.8 cm) and horn girth (11.8 cm). Also, it

should be added that the coefficient of variability for these two parameters was extremely high and for the horn length it amounted 25.55%, while for the horn girth it amounted 14.99%. Such high coefficients of variation for the horn length and horn girth can be explained by the fact that they were caused by small movements of the animals during the measurement.
The values of the body indices in Gatacko cattle are shown in Table 3.

Table 3. The body indices in Gatacko cattle

Indices	Gatacko cattle
Format index (%)	116.13
Chest index (%)	78.54
Compactness index (%)	118.48
Massiveness index (%)	137.58

The format index tells us about the extension of the animal, i.e., whether the format leans towards square or rectangular. In the case when the value of format index is higher than the value of 100, then we are talking about a more rectangular format, as it is the case in this research (116.13%), i.e., these values can be found in animals that came to a standstill during the intrauterine period due to inadequate increase in height (*Lalovic* and *Zdralic*, 2018). Based on the chest index, the constitutional type (qualification) of the animal (population) can be perceived. In our research, this index amounts 78.54% and thus leans towards the digestive type of constitution. The compactness index is one of the most important indicators of an animal physical development. Somewhat higher values are more desirable when it comes to the mentioned body index. In our research, it amounts to 118.48%, which represents a favorable relationship between chest girth and body length. The massiveness index is quite emphasized in this research (137.58%). When the massiveness index amounts than 130%, then we know it's the case of more massive, slightly heavier animals.

Correlational relationships of average values of examined body dimensions as well as statistical significance of correlation coefficients are shown in Table 4.

Height at withers is positively correlated with all examined parameters, statistically confirmed at the level of $P < 0.001$, i.e., $P < 0.01$ for chest width, back shin girth, head length, horn length. Back height is in a highly positive correlation ($P < 0.001$), i.e., high ($P < 0.01$) with most of the examined body dimensions. Correlational interrelations between the back height and the rear shin girth, head length, horn length are positive and by significance confirmed at the level of $P < 0.05$. The same can be concluded for the interrelation of the loin height to the parameters examined in this research. Research by *Nikitovic et al*. (2021) obtained similar values and significances, with statistical significance absent in the interrelation between back height to back shin girth and horn girth.

Body length is statistically, significantly associated to all parameters, at all three levels of significance, with statistical significance lacking in two parameters measured on the head (horn length and horn girth). In the research by *Nikitovic et al.* (2021) statistical significance was absent only to the horn girth. By direction,

all correlation coefficients, so far mentioned by parameters, are positive. Chest width is correlated, at the level of significance P < 0.01, to most of the examined parameters (chest depth, chest girth, pin bones width, forehead width) and is positive in direction.

Table 4. Phenotypic correlations of morphological characteristics in Gatacko cattle

	BH	LH	BL	CW	CD	CG	HW	PBW	FSG	BSG	HL	FW	HNL	HG
WH	.949***	.933***	.629***	.309**	.304**	.709***	.651***	.419***	.420***	.299**	.287**	.463***	.264**	.331**
BH		.898***	.674***	.340**	.353**	.693***	.611***	.385***	.356**	.250*	.218*	.493***	.258*	.276**
LH			.693***	.203*	.209*	.664***	.679***	.368**	.464***	.354**	.388***	.301**	.277**	.323**
BL				.211*	.354**	.575***	.687***	.316**	.256*	.224*	.327**	.440***	.160^{ns}	.121^{ns}
CW					.315**	.328**	.114^{ns}	.333**	-.087^{ns}	-.109^{ns}	-.185^{ns}	.370**	.162^{ns}	.070^{ns}
CD						.221*	.243*	.297**	-.115^{ns}	-.094^{ns}	-.119^{ns}	.466***	-.053^{ns}	.045^{ns}
CG							.487***	.402***	.278**	.148^{ns}	.058^{ns}	.335**	.144^{ns}	.142^{ns}
HW								.385***	.431***	.371**	.441***	.503***	.164^{ns}	.188^{ns}
PBW									.182^{ns}	.106^{ns}	.133^{ns}	.417***	.149^{ns}	.336**
FSG										.912***	.438***	.162^{ns}	.218*	.535***
BSG											.420***	.115^{ns}	.048^{ns}	.409***
HL												.175^{ns}	.270**	.242*
FL													.188^{ns}	.158^{ns}
HNL														.266**

^{ns}P>0.05; *P<0.05; **P<0.01; ***P<0.001

A negative correlation was recorded in the interrelation between chest width, front and back shin girth, as well as head length. The calculated coefficients were not statistically confirmed (P > 0.05). As in the relation of body length to horn length and horn girth, the relation of the same parameters to chest width did not show statistical significance of the calculated parameters (P> 0.05). Correlation coefficients calculated in the interrelation between chest depth and examined parameters were statistically confirmed at the levels of P < 0.001 (forehead width), P < 0.01 (pin bones width), P < 0.05 (chest girth, loin width), while for other parameters are different in direction and statistically unconfirmed. Chest girth is positive in correlation, statistically significant at two levels of significance, P < 0.001 (loin width, pin bones width), P <0.01 (front shin girth, forehead width). The pin bones width is in the statistically significant correlation at the level of P < 0.001 for the forehead width and P < 0.01 for the horn girth, while the statistical significance was not confirmed in relation to other parameters. The front shin girth is highly correlated (P <0.001) to the back shin girth, the head length and the horn girth, i.e., significant at the level of P < 0.05 to the horn length. The back shin girth is in a very high correlation (P <0.001) to head length and horn girth, while the calculated correlation coefficients between forehead width and parameters are not statistically confirmed (P > 0.05), as found in the research by *Nikitovic et al.* (2021). The interrelation between the horn length and horn girth is statistically significant at the level of P < 0.01.

CONCLUSIONS

For the purpose of morphometric characterization of Gatacko cattle, in the Gacko municipality, Republic of Srpska (B&H), phenotypic measurements were performed on a total of 92 necks/individuals of different age categories. The aim

of this research was to analyze phenotypic (morphometric) parameters (15 in total), separated into two logical groups of parameters by region (11 + 4), a total of 92 necks/individuals of autochthonous breed Gatacko cattle (Gacko municipality, B&H – Republic of Srpska). For the purpose of data analysis, the variability of average values of the examined parameters, body indices, correlation of the investigated parameters and the significance of the calculated correlation coefficients were determined.

An unavoidable procedure for phenotypic characterization of a race is morphometric characterization. Knowledge on racial characteristics is necessary while making decisions on race development and breeding programs (FAO, 2012). Furthermore, we can say that the contribution of these researches lies in the continuation of previous researches on morphometric parameters in autochthonous cattle breed (Gatacko cattle), which further provides material for research towards genotyping and establishing the breeding goal.

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LAND COVER AND CARBON STORAGE IN A CERTIFIED SUSTAINABLE COMMUNITY FOREST IN SUMBEREJO VILLAGE, WONOGIRI, CENTRAL JAVA, USING LANDSAT DATA SERIES 2000, 2015 AND 2020

SUMMARY

The Indonesian Ecolabel Institute certified Sumberejo Village's 426.19 hectares of community forest as the first to receive a certificate of sustainable community forest management in 2004 for the first 15 years until 2019. Some economic, socio-cultural, and ecological aspects of this community forest management have been studied, but not the extent of land cover and the amount of carbon storage capacity. Indeed, this data is crucial in determining the role of certified community forests in climate change mitigation. Therefore, the purpose of this study is to look at the changes in land cover and the amount of carbon storage in Sumberejo Village's community forest as a result of certification. Landsat 7 satellite images from the year 2000, Landsat 8 satellite images from 2015, and Landsat 8 satellite images from the year 2020 were used to represent the state-of-the-art community forest before, during, and the end of the certification period, respectively. Using a combination of the forest canopy density model and carbon storage conversion at the national level, we analyzed land cover classes from 2000 to 2015 and from 2015 to 2020, representing changes in the initial and final phases. The SPOT image 2020 land cover classification was then used as training data for a supervised classification-

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maximum likelihood algorithm to classify the images for 2000, 2015, and 2020. The result showed that moderately dense forest dominated the investigated area in 2000, followed by open forest and high dense forest, with 398.58 ha, 83.53 ha, and 35.53 ha, respectively. Total Carbon storage 45,230.02 tons C during this period. In 2015, moderately dense forest increased by 516.63 ha, while open forest significantly decreased by 4.73 ha as a result of tree planting activity, and high dense forest decreased by 1.69 ha as a result of harvesting. Due to public awareness of the need to manage and conserve forests through methodical harvesting, the composition of land cover, as well as carbon storage, remained unchanged in 2020. This consistent condition of carbon storage ensures that the certification has a positive impact on climate change mitigation.

Keywords: Climate change mitigation; Canopy density model; Nationally carbon conversion; Indonesian Ecolabel Institute

INTRODUCTION

After Brazil and the Congo, Indonesia's tropical forests play an important role as the world's third-largest biodiversity site. Furthermore, tropical forests in Indonesia are one of the world's climate regulators, absorbing CO_2 gas from the atmosphere through photosynthetic processes and storing it as biomass (Arianasari et al., 2021); Nurrochmat and Abdulah, 2017). The role of forests as carbon sinks and stores is critical in mitigating the effects of greenhouse gases (GHGs) that cause global warming (Windarni et al., 2018). However, primary tropical forests in Indonesia have suffered massive degradation and deforestation. Between 2001 and 2019, Indonesia's tropical forests shrank by 9.5 million hectares (Butler, 2020). Illegal logging, forest fires, mining, and the transfer of forest functions to agricultural land are all factors that contribute to forest degradation and deforestation (Askar et al., 2018; Wahyuni and Suranto, 2021). Moreover, the high rate of forest destruction as a result of deforestation and forest degradation has drawn international attention. This is due to the fact that the issue not only causes forests loss in Indonesia but it also causes in an increase in GHGs emissions, which eventually leads to the accumulation of GHGs in the atmosphere. Land use, land-use change, and forestry (LULUCF) are the primary contributors to CO_2 emissions in Indonesia (Askar et al., 2018).

During the G-20 summit in Pittsburgh, Pennsylvania, USA, the Indonesian government addressed this critical issue. At the meeting, Indonesia pledged to cut GHGs emissions by 26% on its own, or 41% with international assistance, by 2020 (Bappenas, 2011). One of the Indonesian government's efforts to reduce GHGs is the issuance of Presidential Regulation No. 61 of 2011, which establishes a national action plan to reduce GHGs emissions in Indonesia. The regulation's action plan includes forest and land fire control, network and water systems management, forest and land rehabilitation, industrial crop forest development, private forest development, eradication of illegal logging, deforestation prevention, and community empowerment

(Arupa, 2014). One of the national action plans expected to play a significant role in reducing GHGs emissions is the development of private forests. A private forest, according to the Indonesian Ministry of Environment and Forestry, is defined as a forest that grows on land subject to property and other rights, has a minimum area of 0.25 ha, and is more than 50% devoid of timber crops and other plants (Fujiwara et al., 2018; Hardjanto and Patabang, 2019; Kurniawan et al., 2020). Additionally, experts in some related literature define private forests as forests that grow on property-rights-protected land and are composed of woody trees grown monoculture or in mixed stands, both self-planted and with government assistance (Kurniawan et al., 2020).

Private forests are one type of community involvement that helps to mitigate climate change by absorbing and storing CO_2 in crops. According to Askar et al. (2018); Ivando et al. (2019), private forests can be relied on to reduce GHGs emissions due to their ability to absorb and store CO_2. However, one of the major challenges in maximizing the role of private forests is ensuring the sustainability of private forest management (Kurniawan et al., 2020). Therefore, a mechanism capable of overcoming the sustainability issues associated with private forest management in Indonesia is required. The sustainable community-based forest management (SCBFM) certification program is one mechanism that is expected to be able to address these issues (Mindawati et al., 2006).

This certification program has successfully increased awareness, knowledge, and recognition of the concept of forest management, including private forests, by meeting three aspects of sustainable development: economic, social, and ecological aspects (Rametsteiner and Simula, 2003; Yuwono, 2008). In 2004, the Indonesian Ecolabel Institute (LEI) certified the 426.19 hectares of community forest in Sumberejo Village, Wonogiri Subdistrict, as the first private forest to have received a certificate of sustainable community forest management (Yuwono, 2008). Several studies have themes related to sustainable community forest management in Sumberejo Village, including people's perception of the SCBFM program (Yuwono, 2008), private forest management performance (Anen, 2017), the history of development and acquisition of private forest ecolabel certification (Purwanto, 2015), gender-based private forest management (Kunretno, 2013), farmers' local wisdom in rehabilitating critical land in Sumberejo Village (Ekawati, 2006), financial analysis of private forest farming on several broad strata of land ownership in Sumberejo Village (Jariyah et al., 2003), and contribution of private forests to farmers' household income and village economy (Ichwandi et al., 2007).

Those researches have been only discussed private forest management in terms of economic, socio-cultural, and ecological in general, with no studies on the extent of land cover and the amount of carbon stored as one of the roles of certified sustainable community forest in Sumberejo Village in climate change mitigation. Factually, data on the extent of land cover and the

amount of carbon stored before and after the certification program is critical for determining how important private forests' roles in mitigating climate change are. This study aims to examine the state of land cover and the amount of carbon stored in private forests before and after certification in Sumberejo Village. This evidence is important as the foundation for continuing the community forest certification program. Furthermore, the finding is expected to be taken into account by stakeholders, especially the central government (The Ministry of Environment and Forestry) and local governments (Wonogiri Regency Regional Government and Central Java Provincial Government), when developing policies to reduce GHGs emissions in the context of climate change mitigation.

MATERIAL AND METHODS

Study area

Sumberejo Village, Batuwarno Subdistrict, Wonogiri District, Central Java Province was the site of this study (Figure 1). The research site was selected with purpose, with private forests in Sumberejo Village being the first private forest in Indonesia to receive sustainable community-based forest management certification from LEI in 2004. This village has a land area of 546 ha and is located between 7°32' and 8°15' South Latitude and from 110°41' to 111°18' East Longitude. This area has an elevation of about 274 meters above sea level and is mostly mountainous with a fairly steep land slope (> 40%), with 55 percent of the land being choppy to hilly and 15 percent flat to choppy.

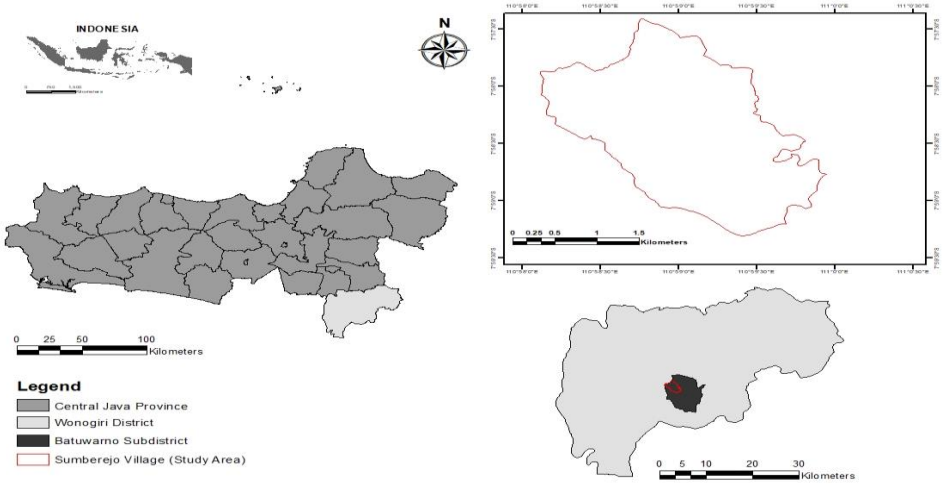


Figure 1. The study area is in Sumberejo Village, Batuwarno Subdistrict, Wonogiri Regency, Central Java Province, Indonesia

Geographical conditions and geological structures with layered/folded limestone have created the impression that this area is earthy rock. The topography is undulating and hilly, with layered limestone dominating the soil structure. In addition, this area is dominated by the association of a soil type of

Mediterranean acid brown lithosol made from a parent of medier tufvolcan with volcanic physiography and hill folds. Furthermore, the solum soil is very thin, with only a small amount of soil visible on the rock's sidelines. Sumberejo Village has a dry climate, with an average annual rainfall of 2,108 mm and 160 rainy days per year. Drought is frequently caused in this area by the uneven distribution of rain and low rainfall.

Data collection and image pre-processing

Satellite imageries from the multi-temporal Landsat 7 (ETM) year 2020 and Landsat 8 (OLI) year 2015 and 2000 were downloaded from the United States Geological Survey (USGS) - Earth Explorer website. Each image used in this study was chosen with the base overcast cover, high deception of the scene, highest satellite picture quality, and accessibility in mind (Emran et al., 2016). Other data used include the Sumberejo Village vector boundary, SPOT images for 2020, and Google Earth images from 2021 (Table 1).

Table 1. The data collected, the date of acquisition, and the sources

Data	Acquisition Date	Sources
Landsat 8, Path/Row 119/65, spatial resolution 30 m	23/08/2020	USGS[1]
Landsat 8, Path/Row 119/65, spatial resolution 30 m	25/07/2015	USGS[1]
Landsat 7, Path/Row 119/65, spatial resolution 30 m	09/09/2000	USGS[1]
Google Earth Image	2021	Google Earth
SPOT Image	2020	LAPAN[2]
Sumberejo Village Boundary	2021	BIG[3]

[1]USGS= United States Geological Survey, [2]LAPAN= Lembaga Antariksa dan Penerbangan Nasional / National Aeronautics and Space Administration, [3]BIG = Badan Informasi Geospasial / Geospatial Information Agency.

All satellite images are USGS L1T results automatically referred to and geometrically corrected to the World Geodetic System (WGS84) datum (Storey et al., 2014). The images are projected in GeoTiff format using the Universal Transverse Mercator framework (zone UTM 49 South). Then, as suggested by Young et al. (2017), radiometric corrections are made using the open-source software Quantum GIS (QGIS) to reduce atmospheric effects that may interfere with data processing. Every one of the seven groups of Landsat images was converted to BIL format before being processed by the FCD Mapper Ver.2 program, with supports from CEOS, TIFF/GEO TIFF, and BMP or BSQ/BIL format. Landsat images can be reprocessed to reduce commotion, for example, atmospheric, water, cloud, cloud shadow, and slope shadow impacts using the pass 1 and 2 steps on Forest Canopy Density (FCD) Ver.2 as proposed by Rikimaru et al. (2002). We can distinguish and exclude the appearance of water, cloud, cloud shadow, slope shadow, and atmospheric effects such as haze and cloud-free mosaic in satellite images by using noise-reduction normalization in

FCD Mapper Ver.2. Finally, we limit the coverage of satellite imagery to Sumberejo Village's vector boundaries.

Land cover classification, land cover changes, and carbon stock analysis

In terms of land cover classification, we used the National Standardization Agency's (Badan Sandardisasi Nasional, BSN) land cover change order framework: SNI-Standard Nasional Indonesia No. 7645-2010. Therefore, we divided the land cover class into four classes based on FCD Mapper Ver. 2, namely non-forest (<10%), open forest (10–40%), moderately dense forest (40–70%), and dense forest (>70%). In addition, this study focuses on two critical issues: deforestation and degradation. Deforestation is defined as a change in land cover from forest to non-forest and open forest, whereas degradation is defined as a change from dense forest to moderately dense forest. We use the method developed by Garai et al. (2018) to calculate the percentage of LULCC using the following formula (Equation 1):

$$CP = \frac{*PLULCA - PLULCA}{PLULCA} \text{x} 100\ \% \quad [1]$$

Where:
CP = Change in percentage (%)
*PLULCA = Present Land Use and Land Changes Area
PLULCA = Previous Land Use and Land Changes Area

Moreover, we recognized land use and determined the spaces of each land cover class, and the absolute carbon stock of each land cover class could be assessed using the carbon stock change approach for the national scale of the corresponding land cover class (Tosiani, 2015). In addition, Table 2 depicts the FCD classification at the national level based on land cover class, identified land use, and carbon storage used in this study.

Table 2. Forest canopy density classification based on land cover class, identified land use and carbon storage at the national level

Forest Canopy Density	Land Cover Class	Identified Land Use	Carbon storage (Ton of Carbon ha^{-1})
<10%	Non – forest	Open land	2.5
10 – 40%	Open forest	Mixed dry land agriculture	30.00
40 – 70%	Moderately dense forest	Plantation forest	98.38
>70%	High dense forest	Secondary forest	98.84

Sources: Rikimaru et al. (2002); Sadono et al. (2020); Tosiani (2015).

FCD Mapper Ver.2 was used to analyze tree canopy density in forested land to simplify the land cover classification process. According to Rikimaru et al. (2002), the condition of forest vegetation is assessed based on canopy density. Using this methodology, FCD Mapper Ver.2 computed four records, namely the

Advanced Vegetation Index (AVI), Bare Soil Index (BI), Shadow Index (SI), and Thermal Index (TI). We created an FCD map for 2000, 2015, and 2020 using FCD Mapper Ver.2, which communicated in rate for each pixel. Table 3 shows the important equations and calculations used by the FCD model for the records. All lists and FCD were determined using FCD Mapper Ver.2 programming.

Table 3**.** Formulas and algorithms used to calculate indices in Forest Canopy Density Mapper

Index		Formula or Algorithm
VI		
	NDVI	= (NIR – Red/NIR + Red)
	AVI	= $[NIR \times (256\text{-}Red) \times (NIR - Red) + 1]^{1/3}$, (NIR – Red)>0
	ANVI	= This index is derived from NDVI and AVI by PCA
BI		= [(SWIR1 + Red) – (Blue + NIR) / (SWIR1 + Red) + (Blue + NIR)] × 100 + 100
SI		= $[(256 - Blue) \times (256 - Green) \times (256 - Red)]^{1/3}$
TI		= This index is calibrated from the thermal data band
FD		= This index is calculated from the first principal component of VI and BI
SSI		= This index is calibrated for the forested land
FCD		= $(VD \times SSI + 1)^{1/2} - 1$

Note: Landsat bands: Visible bands = Blue, Green, Red; NIR = Near Infrared; SWIR = Shortwave Infrared Indices: VI = Vegetation Index; NDVI = Normalize Difference Vegetation Index; AVI = Advance Vegetation Index; ANVI = Advanced Normalize Vegetation Index; BI = Bare Soil Index; TI = Thermal Index; VD = Vegetation Density; SSI = Scaled Shadow Index; FCD = Forest Canopy Density.
Sources: Mon et al. (2012); Pujiono et al. (2019); Rikimaru et al. (2002).

Later, the training data was compiled from the results of the land cover classification analysis using FCD Mapper Ver.2 and SPOT Images. We used a supervised classification-maximum likelihood classification (MLC) algorithm to classify images for years 2000, 2015, and 2020 based on the training data. To reduce the salt and pepper effect caused by spectral effects variability, post-classification smoothing was performed using a 3 x 3 m – pixel majority filter. Finally, image classification was converted to vector format in order to make measuring the area of each type of land cover classification easier.

Accuracy assessment

The accuracy was determined by comparing each QGIS land cover classification result with Google satellite imagery, previously geotagged data, socio-economic and boundary surveys. If the reference data is incorrect, the assessment findings show that many errors occur during the land cover classification procedure (Negassa et al., 2020). Producer accuracy, as defined in Equation 2, is map correctness from the map maker's perspective (the producer). This is the method by which genuine elements on the ground are frequently accurately displayed on the planned guide or the possibility that a specific land front of space on the ground is named. It is also the number of reference locations precisely separated by the total number of reference locations for that class.

$$PA = \frac{TPC}{TPCR} \quad [2]$$

Where:
PA = Producer Accuracy
TPC = Total number of pixels in classification
TPCR = Total number of pixels in classification from reference data (i.e., total row)

The precision from a user's point of view, as shown in Equation 3, is referred to as user accuracy. The User accuracy essentially tells us to know how frequently the class on the map will be available on the ground. In addition, the commission error is supplemented by the user accuracy, with user accuracy equaling 100% commission error. The user accuracy is determined by dividing the total number of correct classifications for a given class by the total number of rows.

$$UA = \frac{TPC}{TPCR} \quad [3]$$

Where:
UA = User Accuracy
TPC = Total number of pixels in classification
TPCR = Total number of pixels in classification from reference data (i.e., total column)

Furthermore, Equation 4 demonstrates how overall accuracy was used to compute a precision proportion for the entire image across all classes present in the characterized image. Overall accuracy, which determines the extent of pixels accurately ordered, can be used to depict the aggregate accuracy of the map for all the classes.

$$OA = \frac{SDE}{TAP} \quad [4]$$

Where:
OA = Overall Accuracy
SDE = Sum of diagonal elements
TAP = Total number of accuracy sites pixels (total column)

The kappa statistics value represents a percentage of the arrangement of classification and reference data (Mishra et al., 2020; Wang et al., 2012). Cohen (1968) classified kappa values were divided into six categories, ranging from 0 to 1: 0 denoted a low probability of correctness. There was a slight chance of accuracy between 0.10 and 0.20, a fair chance of accuracy between 0.21 and 0.40, a moderate chance of accuracy between 0.41 and 0.60, a substantial chance of accuracy between 0.61 and 0.80, and a nearly perfect chance of accuracy between 0.81 and 0.99. A Kappa accuracy value of 50 % to 90% is regarded as adequate (RSPO, 2017). A value of more than 0.6 Kappa coefficients is considered excellent precision. A Kappa coefficient greater than 0.6 indicates that the translation result is precise enough for remote sensing and that no reevaluation is

necessary. The flowchart in Figure 2 summarizes the methods used to assess changes in forest cover and carbon storage.

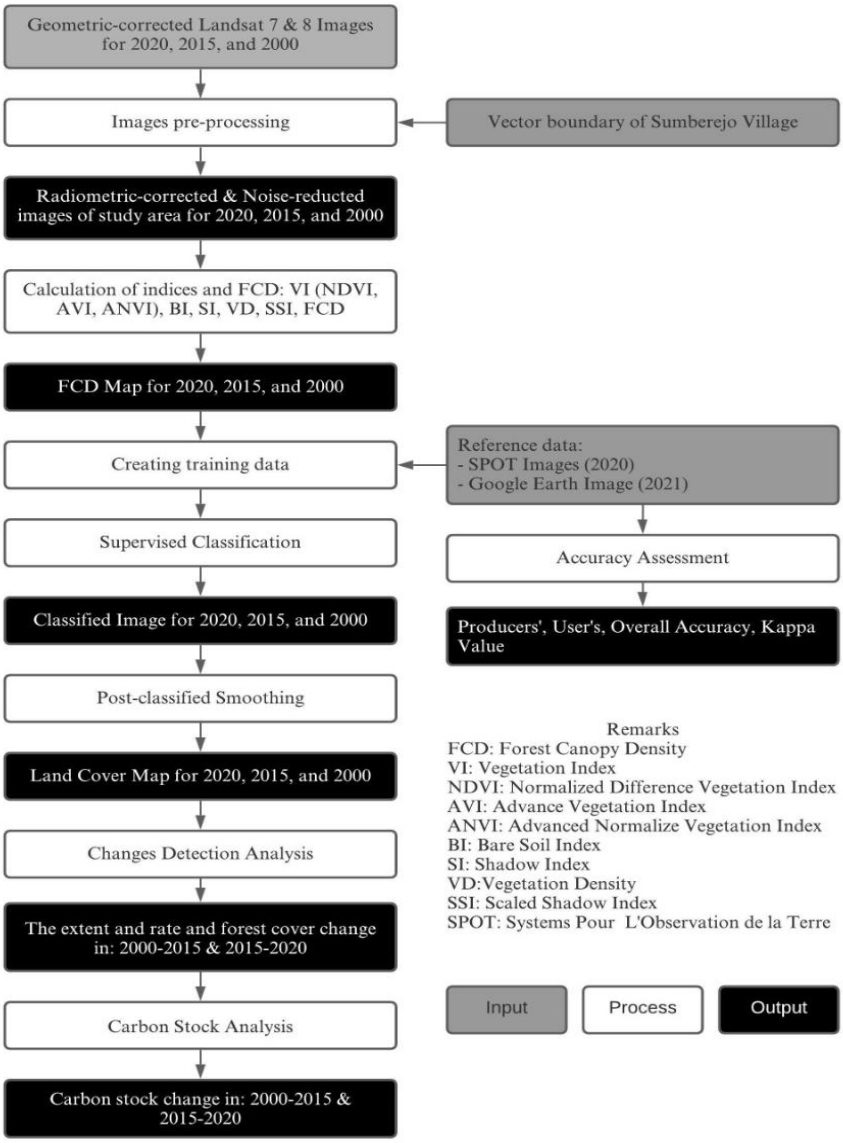


Figure 2. Flowchart of the method used to assess changes in forest cover and carbon storage in Sumberejo Village's community forest

RESULTS AND DISCUSSION

Land cover and changes for the years 2000, 2015 and 2020

Based on the obtained forest land cover, the majority of the areas (77%) consisted of moderately dense forests in 2000, with sporadic open forest areas in some places. Interestingly, this year, there are still many high dense forests,

despite accounting for only about 7% of the total forest area (Figure 3). From 2000 to 2015, the open forest category decreased by 78.80 ha (94.34%) from 83.53 ha to 4.73 ha, and the high dense forest decreased by 33.84 ha (95.24 %) from 35.53 ha to 1.69 ha. In contrast, the area of moderately dense forest increased by 118.05 ha (29.62 %) from 398.58 ha to 516.63 ha. The decline in open forest area between 2000 and 2015 was most likely caused by regional tree planting, as well as an increase in forest stand density over time, transforming the open forest category into a moderately thick forest category. The opposite situation occurred in the high dense forest category, with the area of this category decreasing by 95.24 % between 2000 and 2015 due to a high level of tree harvesting activity in the high dense forest category by the community, resulting in the high dense forest transforming from a high dense forest to moderately dense forest (Figure 4).

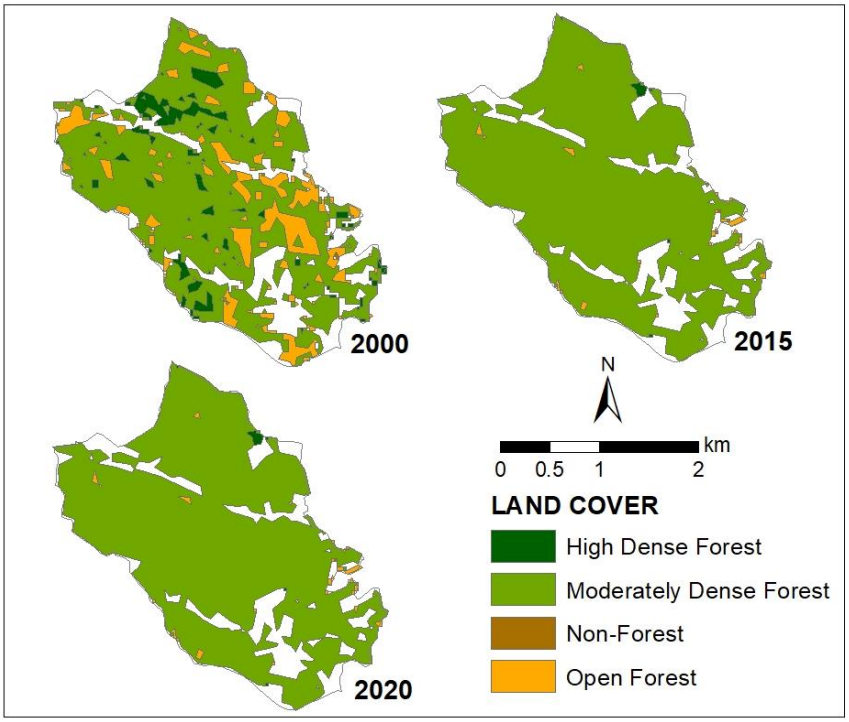


Figure 3. Changes in land cover in the investigated area of Sumberejo Village's community forest from the years 2000 on the left, 2015 on the middle, and 2020 on the right

In 2015, the condition of the forest changed over time. This year, the Sumberejo Village community forest is more dominated by moderately dense forest, accounting for 99% of the total area. This condition shows that after certification, forest density in Sumberejo Village community forest tends to be uniform. It is because there is a possibility that the community will harvest and

replant the forest at the same time. There are several places in the form of open forest and high dense forest with a fairly small area, which may be influenced by the level of community need to sell wood.

In the meantime, except for non-forest classes, there was no change in forest land cover categories between 2015 and 2020. This situation arose as a result of public awareness of the importance of maintaining and preserving forest stands through planned harvesting in order to avoid changing the forest land cover category. Meanwhile, the shift in the non-forest class was caused by the non-forest class's tree planting.

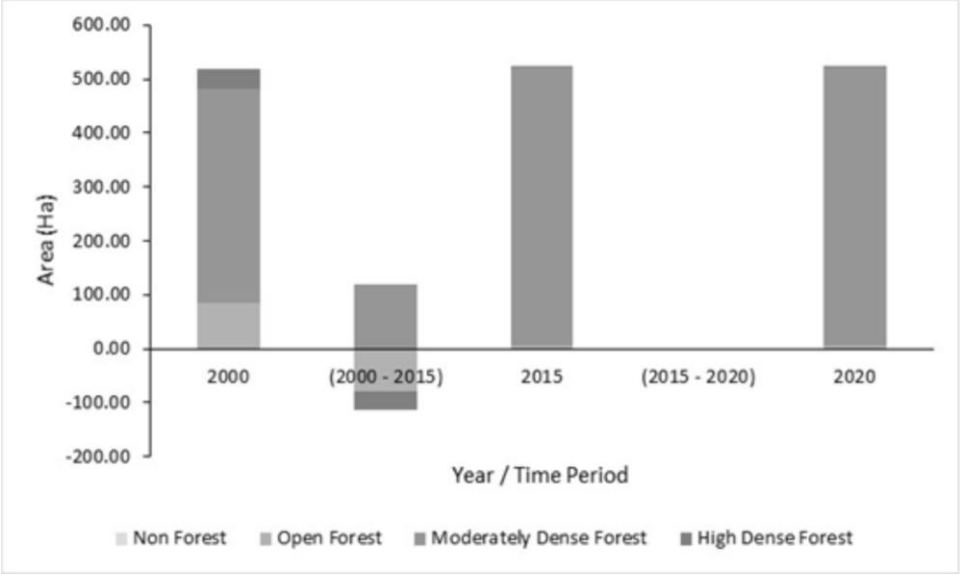


Figure 4. Stacked histogram of land cover and its changes in the investigated area of Sumberejo Village's community forest for the periods of 2000–2015 and 2015–2020

Carbon storage and changes for the years 2000, 2015, and 2020

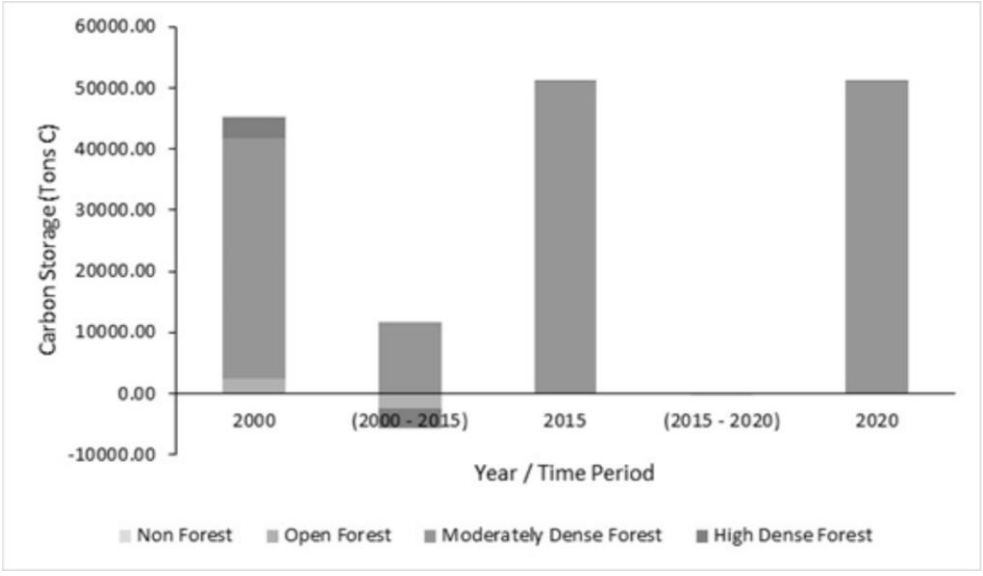
Table 4 and Figure 5 show the carbon storage levels for the years 2000, 2015, and 2020, with the amount of carbon storage stated in Tons C. The highest quantity of carbon storage in 2000 was found in moderately dense forest (39212.09), followed by high dense forest (3511.41) and open forest (2505.98). Moreover, the activities of forest certification started in 2004 caused the increase of moderately dense forest, about 11614.21 tons, and decreased was occurred in dense forest -3344.29 followed by open forest -2364.49 and non-forest -0.09 for the year of 2000-2015. In line with that, the moderated dense forest 50826.30 was increased and followed by high dense forest 167.12, open forest 141.98, and non-forest 0.45 in 2015. During the 2015-2020 period, the carbon storage of non-forest was decreased 0.23 ton, and the carbon storage quantity remained the same for the rest of the forest types. In 2020, the carbon storage status of all forests

except the non-forest remained the same. However, the amount of carbon stored in non-forest areas was reduced and is now 0.23 tons.

Table 4. Carbon storage and changes in the investigated area of Sumberejo Village's community forest for the years 2000, 2015, and 2020

Land Cover Classes	Carbon stock (Tons C)		
	2000	2015	2020
Non-Forest	0.54	0.45	0.23
Open Forest	2,505.98	141.89	141.89
Moderately Dense Forest	39,212.09	50,826.30	50,826.30
High Dense Forest	3,511.41	167.12	167.12

Figure 5. Stacked histogram of carbon storage and its changes in the investigated area of Sumberejo Village's community forest for the time periods of 2000–2015 and 2015–2020



The certified sustainable community forest in Sumberejo village exhibits highly effective in the growth of carbon storage. Therefore, forest certification operations have a positive impact on the enhancement of forest carbon storage (Bettinger et al., 2017). The Sumberejo Village community forest, particularly the open forest space, has shrunk as a result of the locals collectively began planting more trees, resulting in a more fairly wooded forest region. Planting more trees, also known as afforestation and reforestation, could help to increase live-tree carbon storage in forests and carbon buildup in soils, as well as expand forestland and provide a variety of ecological services (Domke et al., 2020). In this case, increasing carbon storage was extremely beneficial in reducing carbon emissions, which caused climate change (Effendi, 2012). In the previous study conducted by Ulumuddin et al. (2005), developing countries received investment funding from

industrialized countries to support programs that would reduce emissions, such as forestry projects that included activities to promote atmospheric carbon absorption. These activities were mostly carried out by expanding forest areas or preventing deforestation.

Furthermore, as the findings of this study showed, forest certification had primarily positive effects on the environment and society. However, Girolami and Arts (2018) found that certified harvest had a detrimental effect on biomass and tree carbon storage. When compared to pre-harvest reconstructed conditions, biomass was decreased by one-third, lowering potential commercial carbon storage values by 25-30%. In addition, Blackman et al. (2015) reported that Forest Stewardship Council (FSC) certification had a negative impact when compared to pre-harvest reconstructed stands, but not when compared to non-certified stands; hence the negative impact was moderate, which was only -0.50. Moreover, the forest certification in the private forest in Sumberejo village might have gained the same contribution as the previous study. In comparison to non-certified areas, FSC certification did not lower carbon emissions from logging activities. In line with that, another study revealed that FSC had no statistically meaningful impact on deforestation rates in forest management units in Mexico. The threshold of influence was set at 0 for this inquiry (Blackman et al., 2015).

CONCLUSIONS

Forest land-use changes in forest land use, such as forest degradation and deforestation, are significant contributors to carbon emissions and climate change. As a result, improving and maintaining forest land cover not only helps to mitigate the effects of global warming and climate change but also helps to improve societal and environmental services. Furthermore, obtaining forest certification is the most important thing to emphasize to improve forest land cover.

The amount of carbon stored in community forest woods in Sumberejo Village increased significantly between before and after certification. Between 2000 and 2015, land cover shifted from open forest and extremely thick forest cover classes to moderately dense forest cover classes, most likely as a result of increased forest density. Meanwhile, due to public awareness of the need to manage and conserve forests through methodical harvesting, there was no change in forest cover from 2015 to 2020. Changes in forest land cover had an impact on carbon storage in Sumberejo Village community forest woods, as predicted by forest land cover results.

To summarize, it is strongly recommended that additional private woods be certified in order to combat and mitigate the effects of global warming and climate change, as the residents have performed at the study site in a certified community forest of Sumberejo Village, which would increase the area's production and improve land cover. Forest certification has the potential to increase carbon absorption while also having a significant positive impact on climate change mitigation.

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CLIMATE CHANGE AND DECISION SUPPORT USING THE COMPUTER TOOL INSTAT FOR EL GANZRA REGION, MOROCCO

SUMMARY

If the scarcity of water resources and their limitation is a characteristic of the climate of Morocco, this phenomenon seems to know a distinct accentuation during the last decades. But, on the other hand, the scarcity of this resource and the drought are the main constraints of agricultural production; the situation then is more alarming. Although scientists in the region have developed many technologies to cope with these environmental problems, the difference between the yields achievable by farmers and the potential yields generally remains huge in rainfed and irrigated areas.

This paper develops the problem of rainfed agriculture in the Khemisset region, El Ganzra site, in the constraining context of climate change and natural resources degradation. Indeed, several measures are necessary to cope with these climate changes; therefore, we propose a method of agro-climatic analysis of the first significant rains. Our investigations, which are part of the decision support approach, are based on choosing the suitable period for sowing cereals using the "InStat" tool. The method used is based on a quantitative approach, allowing us to study the Spatio-temporal variability of precipitation and the analysis of drought intensity on the site. Consequently, the modelling makes it possible to propose scenarios to farmers regarding sowing dates and crop selection at the appropriate time.

Keywords: rainfed agriculture, drought, adaptation, InStat, sowing cereals, climate change, El Ganzra.

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INTRODUCTION

Climate change (CC) is one of the most pressing challenges facing humanity in the Anthropocene era (El Bilali *et al.*, 2020). CC in recent decades is a major global problem and therefore solutions are being sought to mitigate its consequences. Climate change can be manifested as a change in climate elements relative to average values or as a change in the distribution of climate events relative to average values (Simunic *et al.*, 2021).

The Intergovernmental Panel on Climate Change (IPCC) stated in 2008 that natural resources and agriculture are among the systems and sectors that are particularly vulnerable to climate change and will be strongly impacted. Globally, while average precipitation is expected to change slightly, variability is expected to increase. As a result, extreme weather events, temperature, and precipitation are expected to occur more often.

Morocco is a country of contrasting landscapes and climate risks due to a very high spatial and temporal heterogeneity of climate. Climate change will undoubtedly have essential consequences in agriculture on crop yields, the distribution range of cultivated species, irrigation water availability, and ecosystems' fragility. Climate projections (Ait-El-Mokhtar *et al*., 2022) indicate that aridity will progressively extend due to decreasing rainfall and increasing temperature. However, climatic hazards make agricultural production conditions increasingly difficult (Aziz, 2022). Therefore, the production loss proves to be high and not negligible as it is predicted to obtain between 5.5 and 8.0% reduction in grain yield (Balaghi *et al*., 2010). Consequently, climate change will exacerbate the degradation of resources vital to agriculture, mainly water, soil, and agrobiodiversity. Thus, climate change will exacerbate the degradation of resources vital to agriculture, mainly water, soil, and agrobiodiversity.

This climate change impacts human activities and cropping systems. The biological relationship between harvesting and climatic conditions makes agriculture the first activity affected by this phenomenon. This impact differs from one area to another, and the consequences are not felt in the same way throughout the world (Chakirou, 2005). Gommes *et al*. (2009) showed that climate change would negatively affect Moroccan agriculture in the coming decades. Due to climate change, these impacts will be particularly pronounced in rainfed agriculture, where most small farms are concentrated. This is essentially subsistence family farming, with low resilience to climate change.

Since 2013 Morocco has continued the development of its national environmental legal framework apace, with major updates to its primary legislation on water (Law No. 36-15 published in 2016), the coastal zone (Law No. 81-12, of 2015), and on the environmental assessment (Law No. 49-17, passed in July 2020). New laws on waste, climate change, access to genetic resources and the fair and equitable sharing of the benefits arising from their use are being developed. A swathe of regulatory improvements is being considered, with laws being drafted, including for protected areas and climate change. The law’s purpose is to: (a) Strengthen the protection and conservation of resources

and the natural environment, biodiversity and cultural heritage and prevent and combat pollution; (b) Integrate sustainable development into sectoral public policies and adopt a national strategy for sustainable development; (c) Harmonize the national legal framework with international conventions and standards; (d) Strengthen measures to mitigate and adapt to climate change and to combat desertification; (e) Decide on institutional, economic, financial and cultural reforms in environmental governance; (f) Define stakeholder commitments in matters of environmental protection and sustainable development; (g) Establish an environmental responsibility regime and an environmental control system.

Specifically, in terms of legal reforms, the law foresees, among other developments: (1) The adaptation of the water legislation to the requirements of sustainable development and the combined effects of desertification and climate change; (2) The adoption of a targeted legal regime for the protection of soils against degradation and pollution and for optimal land use; (3) The establishment of an environmental liability regime (UNECE, 2021).

In this study, we will analyze the situation of the EL Kansera (El Ganzra) site, which is undergoing the same changes and shows increased vulnerability to CC. These conditions require better water resources management by implementing adequate adaptation strategies while allowing better planning of their actions. The study is original and the knowledge on spatial and temporal characterization of the climate is necessary to provide as technical solutions for this region. The annual rainfall received in this semi-arid region is highly variable and experiences significant fluctuations in time and space. Periods of water deficits of varying lengths can occur at any time. In this region, droughts at the end of the cycle are the most frequent, while those in the middle are rare but the most dangerous. The climatic monitoring of the agricultural season (the choice of the sowing date is a delicate decision) allows the formulation of alternative crop management proposals according to different rainfall deficit scenarios. This is only possible with a good knowledge of this environment, the characterization of the climate, and the identification of recent changes based on appropriate techniques.

MATERIAL AND METHODS

Morocco is located in North Africa and has a land area of 446,550 km². The country has a high variety of elevation from the lowest point of Sebkha Tah, 55 metres below sea level, to the highest point of Jebel Toubkal which rises to 4,165 metres. A large part of Morocco is mountainous. The Atlas Mountains are with the direction from the southwest to the northeast and are mainly located in the centre and south of the country and form a backbone of the country. The Rif Mountains are located in the North, stretching from the northwest to the northeast over the region bordering the Mediterranean Sea. Most of the southeast portion of the country is the sparsely populated Sahara Desert (UNECE, 2021).

Along the coast of Mediterranean Sea, the climate is warm, with dry summers and mild winters. Inland, the climate is more severe, getting hotter and more extreme closer to the Sahara Desert.

Figure 1. Detail of a physical space in central part of Morocco *(Photo: Spalevic, December 2017)*

On the Atlantic Ocean coast, Morocco's capital of Rabat has an average January low temperature of 8 ℃ and an average July high temperature of 28 ℃. By contrast, the city of Marrakesh, which is located farther inland, has a January average low of 6 ℃ but a much-elevated average July high temperature of 37 ℃. Average annual precipitation can reach to more than 1,000 mm in the mountainous areas of the North but is less than 300 mm in the basins of the Moulouya, the Tensift, the Souss-Massa; and areas south including the Atlas Mountains and the Saharan zone. Typically, there are two rain periods per year, one in the fall and one in winter. The annual number of rainy days varies from about 30 in the South of the country to near 70 in the North. (UNECE, 2021).

The study area is located 40 km northeast of Khemisset city, the El Ganzra site, which straddles the Sidi Slimane plain, and the pre-Rifian marly hills (34°02'31.6"N 5°55'27.7"W; 34.042117, -5.924363).

This area is characterized by altitude variations (the western part is at 207m, the eastern part is at 259m, 300m in the southern position, and 473m in the northern part). Therefore, we note a north-south and east-west decrease in altitude. The site's climate is semi-arid, and the rainfall and thermal regimes with two distinct seasons: the dry season begins in May and ends in September (5 months), and the wet phase begins in October and ends in April (7 months). The annual rainfall average is 464 mm, and the yearly temperature average varies between 7.4°C to 35.4°C; sometimes, the temperature can reach 46°C.

The study area remains, despite its topography generally uneven and with its important geographical location rich in water resources, it is at the intersection of two basins among the largest in Morocco, Sebou and Bouregrag. Thus, the water table of this site is under the administration of two Water Basin Agencies (ABH): Sebou and Bouregrag. We can also distinguish permanent and temporary or intermittent watercourses. In addition, the most widespread vegetation cover is the reforested forest consisting mainly of secondary species of Eucalyptus trees, and wild olive trees named "Jebbouj". The forest of El Ganzra extends over an

area of 930 ha is an important grazing site for the breeding of forest livestock of secondary species. The main economic activity is agriculture with a useful agricultural area UAA of 15117 ha, of which cereal constitutes 71%, insofar as it employs a significant portion of the population. However, this sector remains highly dependent on climatic hazards.

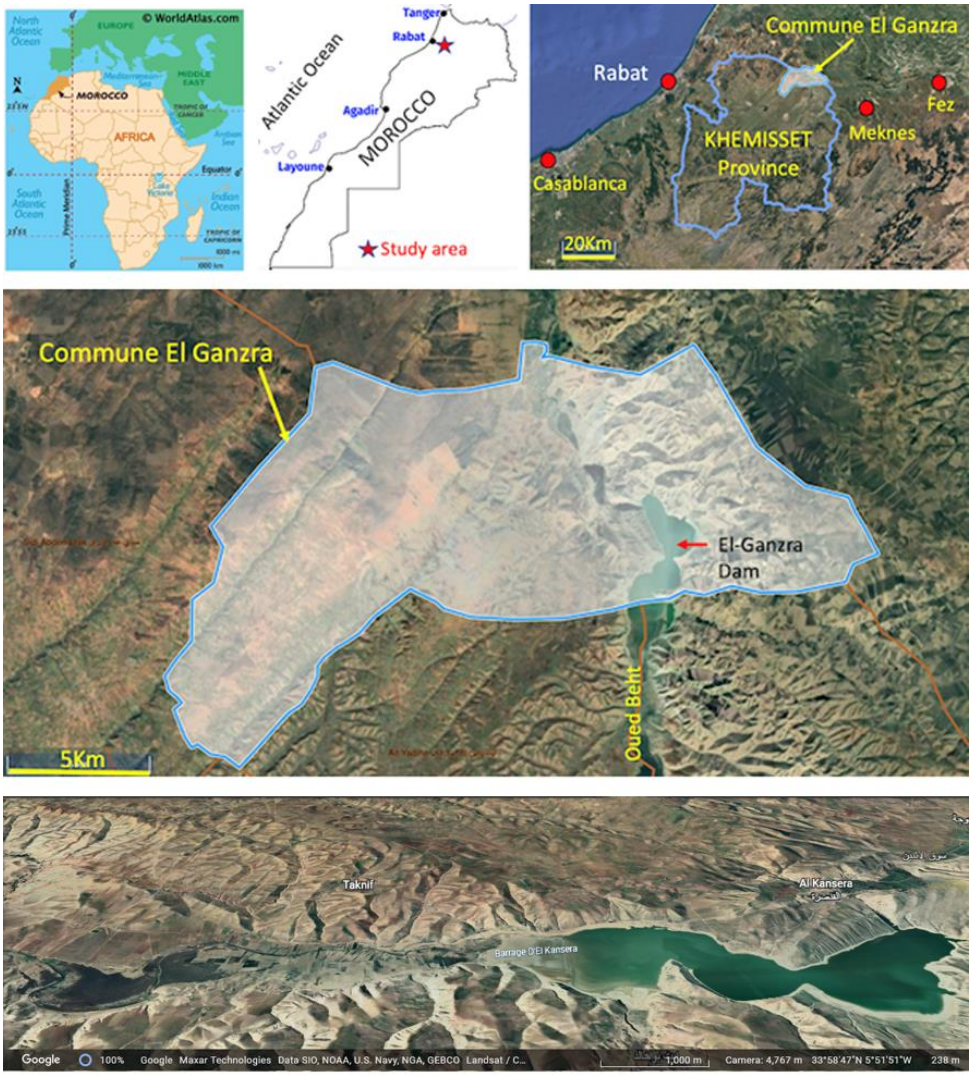

Figure 2. The Study Area
(Source: report, Khemisset Urban Agency, 2016 and Google earth 2022)

Study of climate variability. As far as the climatic data are concerned, they have been obtained from multiple sources (National Meteorological Directorate, Hydraulic Agencies). Our investigation will analyze the available data (rainfall, maximum and minimum temperatures, potential evapotranspiration, and other

related indices) on the El Ganzra region to find and characterize these changes. The rainfall variability will focus mainly on determining the central tendency and dispersion parameters and highlighting the trends.

Figure 3. Panoramic view on the Al Qansra dam (Source: Google)

- Central tendency parameters: The arithmetic mean was calculated over a 76-year series. It is obtained by summing the distinct values observed, each of which is assigned a weight equal to its frequency (Vessereau, 1947; Aston *et al*., 2022). The mean $\overline{X}$ identified the different rainfall rhythms that indicate the trend of decreasing or increasing rainfall; the mean fields identify the surplus and deficit periods. Thus, the analysis of these data allowed us to characterize the rainfall evolution.

- Dispersion parameters: We calculated the dispersion parameters from the mean fundamental central tendency parameter. These dispersion parameters are the standard deviation and the coefficient of variation. The Coefficient of Variation is the most widely used means of testing and quantifying the variability of a reality or a statistical phenomenon. It is the ratio of the standard deviation to the mean, expressed as a percentage (Houndénou, 1999; Vissin, 2007; Lokossou *et al*., 2020). The coefficient of variation is used to compare the degree of variability of rainfall in space. The standard deviation is used to assess the absolute dispersion of values around the central value (Vissin, 2007). However, dispersion parameters alone are not sufficient to measure variability because they do not describe rainfall and hydrometric series (Vissin, 2007). Thus, to better characterize variability, the standardized precipitation index is necessary.

- Standardized Precipitation Index (SPI): From the standard deviation, the Standardized Precipitation Index or (SPI, equation 1) represent the cantered anomalies reduced rainfall inter-annual, was calculated (Bergaoui & Alouini, 2001; Docheshmeh Gorgij *et al*., 2022). The following equation calculates the anomalies:

$$SPI = \frac{(\boldsymbol{Xi - Xm})}{\boldsymbol{Si}} \quad (1)$$

where:
SPI: The Standardized Precipitation Index,
Xm: the average annual rainfall observed for the given statistical series,
Xi: the cumulative rainfall for a given year I,
Si: the standard deviation of the annual rainfall observed for a given statistical series.
Depending on the value of the SPI, a distinction is generally made between positive values (SPI>0) and negative values (SPI<0). We used The SPI to determine the indicators of rainfall variations and specifically the years marked by a rainfall surplus or deficit in the study area.
***- Aridity index*:** This index, sometimes called De Martone's drought index, is the ratio between the mean annual values of precipitation (P) and temperature (T) plus 10°C (De Martonne, 1926). It's defined by the following equation:

$$I = \frac{P}{T+10} \quad (2)$$

where:
T: average annual temperature in °C;
P: mean annual precipitation in mm.

The Aridity index can also be calculated monthly and indicates the degree of dryness with the average evaporative demand of the atmosphere approximated by the temperature. The aridity increases when the value of the index decreases. At the global level, (De Martonne, 1926; El Asri, Larabi and Faouzi, 2022) proposed six significant types of macroclimates ranging from the desert or hyper-arid zones (I < 5) to the humid zones with predominantly forest (I > 40). Exceptional precipitation and other aggregates characterize the hyper-arid zones.

InStat software. The main activity of the population is agriculture, with having either "Bour" or irrigated surfaces under cultivation. Bour agriculture is dependent on natural conditions: rainfall and other climatic factors, particularly temperature and wind. In this study, we simulated the decision-making for an agricultural campaign, based on daily rainfall data of more than thirty years, using the tool "InStat". It is software that allows making the decision for a crop year by processing the climatic data not only rainfall, but it also helps to rationalize its campaign for a better yield. It could also be useful in modelling for the choice of scenarios in terms of sowing dates in the appropriate time (Gizaw *et al*. 2021).

RESULTS AND DISCUSSION

Climate characterization. Agriculture in El Ganzra is rainfed agricultural production. The global evolution of rainfall was analyzed at the station of El Ganzra. The rainfall is, for the subject region and its agriculture, more important in the winter than in the summer period. The average rainfall is 464 mm. The driest months are July and August with only 1 mm. With an average of 81 mm, the month of December has the highest precipitation (Table 1).

Table 1: rainfall recorded at the El Ganzra station (Source: Climat-data.org)

	Jan	Feb.	March	Apr.	May	June	July	August	Sept	Oct.	Nov.	Dec.
Precipitation (mm)	63	55	59	46	26	7	1	1	9	43	73	81

The variation in precipitation between the driest and wettest months is 80 mm. The average annual precipitation is about 430mm. December is the wettest month.

The study area has an average annual temperature of 19.5°C. However, the yearly average temperature is 18°C. The maximum value is in August with a temperature of 35.4°C, and it can reach 46°C. The minimum is 7.4°C (Table 2).

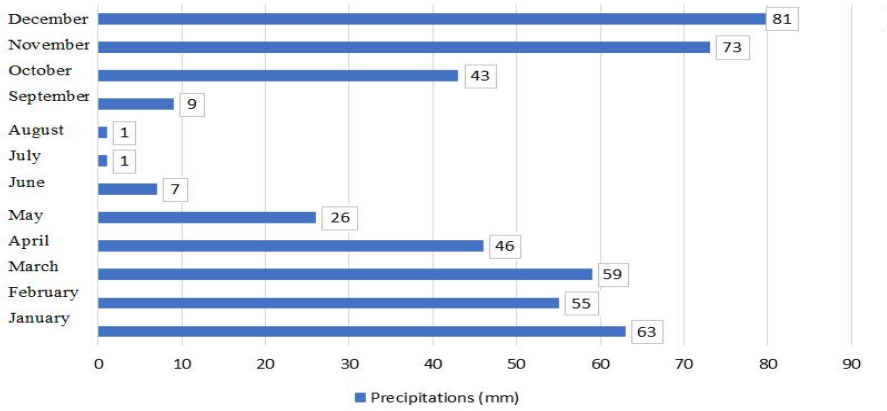

Figure 4: Annual average rainfall average at El Ganzra Station

Table 2: Annual temperature average (min. and max.) at the El Ganzra station

	Jan	Feb.	March	Apr.	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Average temperature (°C)	12.2	13.3	15.7	17.7	20.3	24	26.9	27.6	24.9	21.2	16.5	13.3
Min. temperature (°C)	7.4	7.9	10	11.8	13.7	17.1	19.1	19.8	18	14.9	11.1	8.3
Max. temperature (°C)	17.1	18.8	21.4	23.6	27	30.9	34.8	35.4	31.9	27.5	21.9	18.3

(Source: climate-datat.org)

With preparing an Umbro-thermal Diagram of El Ganzra (Figure 5), there are two phases: wet and dry. Wet period is from October to April (7 months) and the Dry period is from May to September (5 months).

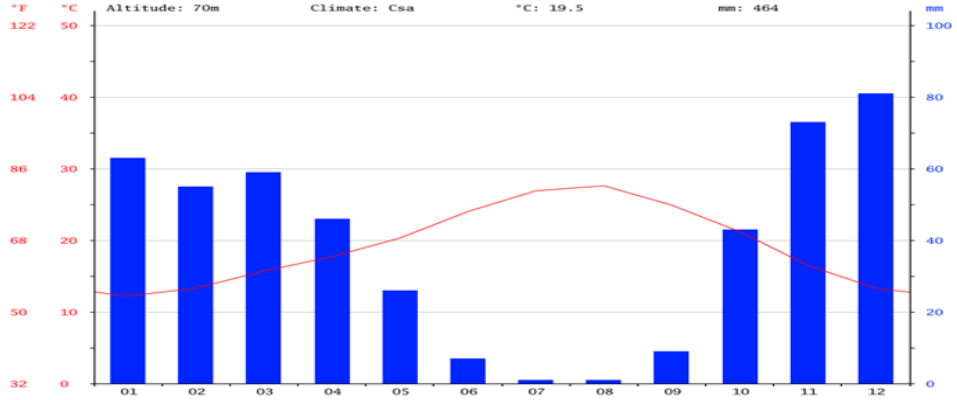

Figure 5: Two phases of dry and wet periods at the El Ganzra Commune

Emberger bioclimatic index. The Emberger bioclimatic index stands out among the bioclimatic indices traditionally used in North Africa and elsewhere in the Mediterranean (Emberger, 1931; Gaussen, 1954; Daget, 1977, and Quezel, 1979). This takes into account the annual precipitation, the average maximum temperature of the warmest month (M in °C) and the average minimum temperature of the coldest month (m in °C) (Emberger, 1955). The position of the study area (El Ganzra) on the Emberger Bioclimatic diagram indicates that this area is under Semi-Arid climatic conditions as presented in the Figure 6. (Siba *et al.*, 2022; Vessella and Schirone, 2022).

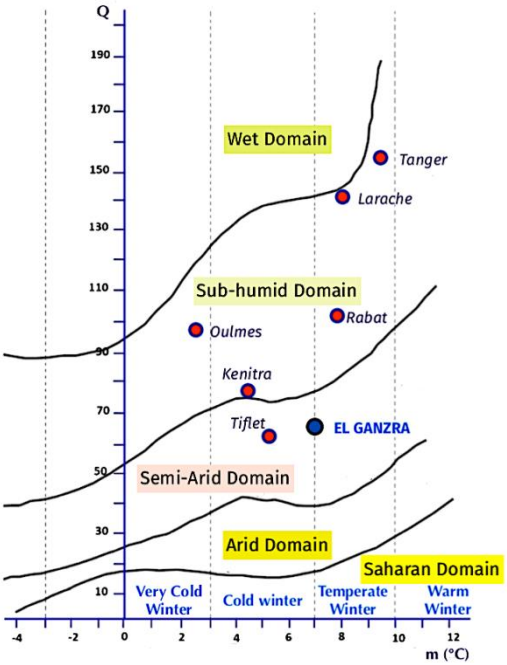

Figure 6: Location of the El Ganzra site on the Emberger diagram

The Emberger bioclimatic index is expressed by the equations 3 and 4, as follow:

$$Q2 = \frac{2000 \times P}{(M+m+546.40) \times (M-m)} = 44.15 \quad (3)$$

Stewart corrected this equation as follow:

$$Q2 = \frac{3.436 \times P}{(M-m)} \quad (4)$$

where:

P: annual rainfall in mm = 364.2mm

M: the maximum temperature of the hottest month in °C usually (July) in our case is August is 35.4°C; m: the minimum temperature of the coldest month in °C (January) is 7.4°C.

For m = 7.4 °C corresponds to a precipitation of 63 mm, then the point of intersection whose coordinates are (7.4, 63) is the semi-arid bioclimatic.

The De Martone's Aridity Index. According to De Martone's table of classes, the Aridity Index of the site El Ganzra is located between 10 and 20 (table 3). Therefore, its climate is semi-arid, and its bioclimatic stage type of climate occupies 25% of the surface of Morocco.(Elyagoubi and Mezrhab, 2022). The De Martone's Aridity Index is (equation 2):

I = 12.36 (with P=364.2 mm, and T=19.47 °C)

Table 3: Importance of climate classes according to the De Martone Aridity Index

Class	Climate Types	% Total Moroccan Area
> 20	Humid and Sub-Humid	11
10 to 20	Semi-Arid	25
5 to 10	Arid	11

Drought intensity. The climate deficit is the second side of the same coin "drought", it is calculated using the following formula:

$$Dc = P - PET \qquad (5)$$

where Dc is Climatic deficit (drought); P is Mean annual precipitation; PET is Potential Evapotranspiration.

Potential evapotranspiration (PET, expressed in mm) is a concept defined by Thornthwaite (1955); and is expressing the quantity of water that would evaporate from the soil and be transpired by the vegetation if there were no water supply problems. It is therefore independent of the actual water availability of the soil. It depends only on the capacity of the ambient atmospheric environment to induce evapotranspiration and thus mainly involves energy indicators. The Thornthwaite TPE formula is defined by the following equation:

$$\text{PET} = 16 \times \left(\frac{10 \times T}{I}\right)^{\alpha} \times K \qquad (6)$$

where PET is Potential evapotranspiration; T is Average temperature of the month considered, expressed in ° C.

To calculate I and α we use the Naoura method (Naoura, 2012, 2021) as follow:

I: The sum of the monthly thermal indices : $I = \sum i = \sum_{i=0} \left(\frac{T}{5}\right)^{1,514}$ (7)

α: Coefficient expressed as a function of the sum of the monthly thermal indices:

$10°C \leq I \leq 80°C$: $\alpha = \left(\frac{1,6 \times I}{100}\right) + 0,5$ (Naoura, 2012) (8)

K: corrective factor depending on the month (m) and the latitude: In our case the result are as follow (table 4):

Table 4: The PET parameters calculation per month

	Jan	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
T°c	12.2	13.3	15.7	17.7	20.3	24	26.9	27.6	24.9	21.2	16.5	13.3
K	0.88	0.85	1.03	1.09	1.2	1.2	1.22	1.16	1.03	0.97	0.87	0.86
I	3.86	4.40	5.65	6.78	8.34	10.75	12.78	13.28	11.37	8.91	6.10	4.40
PET	22.69	26.15	44.50	60.18	87.70	123.53	158.59	158.93	114.32	77.47	41.61	26.46

I = 96.61; a = 2.045784182; PET total = 942.14; PET average = 78.53; (Source: the Charles Warren Thornthwaite formula)
Finally, by using equation (5): DC = 464 – 942.14 = -478.13 mm

Interpretation. The climatic deficit of a value of -478.13 mm per year reflects the degree of dryness, taking into account the evapotranspiration, which also aggravates the already semi-arid climatic situation, which implies that the site el Ganzra is a sunny site and which knows a temperature always high, an important evaporate activity, in spite of its humid microclimate, especially for running or stagnant waters.

Standardized Precipitation Index (SPI). Taking into account the drought that can be studied only in a wide Spatio-temporal extent, in our case we will be interested in a geographically limited site "spatial entity" well determined, but a temporary extent of up to 31 years (with daily, monthly, annual data missing, some of which are calculated by the method of averages and the others have been neglected not to falsify the results).(Cerpa Reyes, Ávila Rangel and Herazo, 2022) We use the following equation:

$$SPI = \frac{Xi - Xm}{Si} \quad (9)$$

where: SPI : Standardized Precipitation Index; Xm : the average annual rainfall observed for the given statistical series; Xi : the cumulative rainfall for a year i, Si : the standard deviation of annual rainfall observed for a given statistical series.

Depending on the value of the SPI, we generally distinguish between positive values (SPI>0) and negative values (SPI<0). SPI classification according to McKee *et al*. (1993) is presented in the Table 4.

Table 4: SPI classification according to McKee *et al*. (1993).

SPI Values	**Characterization**	
>2.0	Extremely wet	Humidity
1.5 to 1.99	Very wet	
1.0 to 1.49	Moderately wet	
−0.99 to +0.99	Normal	**Normal**
−1.0 to −1.49	Moderately dry	Dryness
−1.5 to −1.99	Severely dry	
<−2.0	Extremely dry	

Advantage: Precipitation is the only parameter available. The index can be calculated for various time scales, and it allows early detection of drought situations and assessment of their severity. It is less complex than many other indices, notably the Palmer Drought In decks. **Disadvantage:** Therefore, it only quantifies the precipitation deficit, values based on preliminary data may change, and values will vary if the length of the survey period increases.

Case of the last thirty-one years (31 years). We considered the statistical sequence of precipitation data for the last 30 years (1979-2015), knowing that five years of data are missing, during this period[2]. The results obtained are presented in the Figure 7 and Table 5).

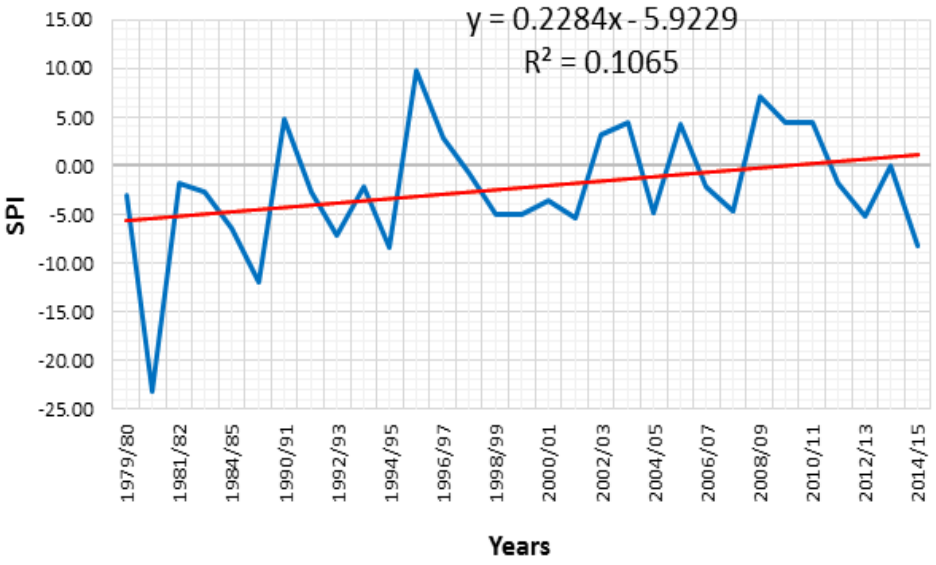

Figure 7: Standardized index of precipitations at El Ganzra station during the last 31 years (source: ABH Sebou)

The Figure 7 shows that the curve with a straight line (trend curve) is with almost zero slopes, which implies a trend for the same negative values.

Table 5: Recapitulation of the type of climate

	SE	SF	SM	HM	HF	HE
SPI	**18**	**2**	**1**	**1**	**0**	**9**
%	**58.06**	**6.45**	**3.23**	**3.23**	**0.00**	**29.03**

Source ABH Sebou

[2] Missing years are : 1982-83 ,1986-87 ,1987-88 ,1988-89, 1989-90.

We noted that the period of drought is 21 years and 11 years of the wet period, i.e., drought is dominant with a frequency of 2 years out of 3 years (64%), which confirms that the site of El Ganzra is of a dry climate, therefore is an arid or semi-arid area. The highest rainfall value was 471.55 mm recorded in 1995-1996, while the minimum value was about 122.90mm recorded in 1980-1981. This is due to the general warming that the globe is experiencing and, consequently, Morocco too. Therefore, the NAO activity (negative) will undoubtedly be the scientific explanation.

The calculation of the climatic deficit, having a consequence of hydric deficit, and the analysis of the drought index indicate that the site knows a meteorological rainfall deficit (i.e., meteorological drought). The latter will indeed impact agriculture, despite the irrigation favoured by the presence of El Ganzra dam. Furthermore, this will generate an agricultural drought, and finally, this deficit will impact the hydrology, and therefore we are facing a hydrological drought.

The site is experiencing a drought that has been raging for years in this region; can we still hope for generous rainfall for a good agricultural season, or can we anticipate and decide on other alternatives? We made a simulation to help us make the right decision using the computer tool "INSTAT" to know precisely when the sowing will start.

Apart from the relatively abundant precipitation, i.e., about 330 mm/year, temperatures can fall to low degrees during the winter months and exceed very high thresholds of 0 to 35°C.

Decision support via "InStat" in the study area. Successful seeding is a prerequisite for better grain production in the fall. Therefore, one of the important indicators affecting final yield is the seeding date. Agronomic research results have shown that early seeding allows the crop to take advantage of the season's first rains because it significantly improves fall grain yields. Indeed, the concept of first significant rainfall (FSR) could help farmers and technicians decide on planting planning. These first significant rains are expressed as the probable date of obtaining for ten successive days an amount of rain that can ensure germination and emergence based on a long series of daily rainfall data. Hence the usefulness of using an agro-climatic analysis method for FSR such as the INSTAT software.

The "InStat" software's principle consists of processing daily rainfall data over a period equal to or greater than 30 years. This processing is done according to scenarios and consists of choosing the best scenario for which the sowing will be profitable or beneficial from October, which remains the ideal month.

Hereafter, we explain the possible scenarios for which there is a cumulative rainfall of 20mm in 10 or 11 successive days. Then, there are at least 1 to 4 days of rain of the said cumulative rainfall.

The first scenario: with 10 days (Figures 8 to 11, and table 6)

Rainfall of 20 mm in at least one day: (Fig. 8)

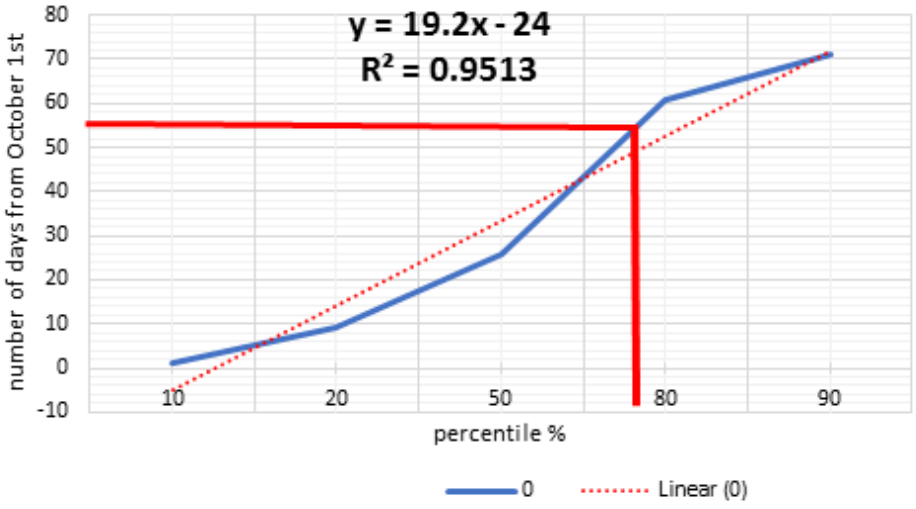

Figure 8: The day of sowing 1st case (1 day)

The value of 75% corresponds to the 55th day from October 1st, so the sowing day is November 24th, the fourth week of November.

Rainfall of 20 mm in at least two days: (Fig. 9)

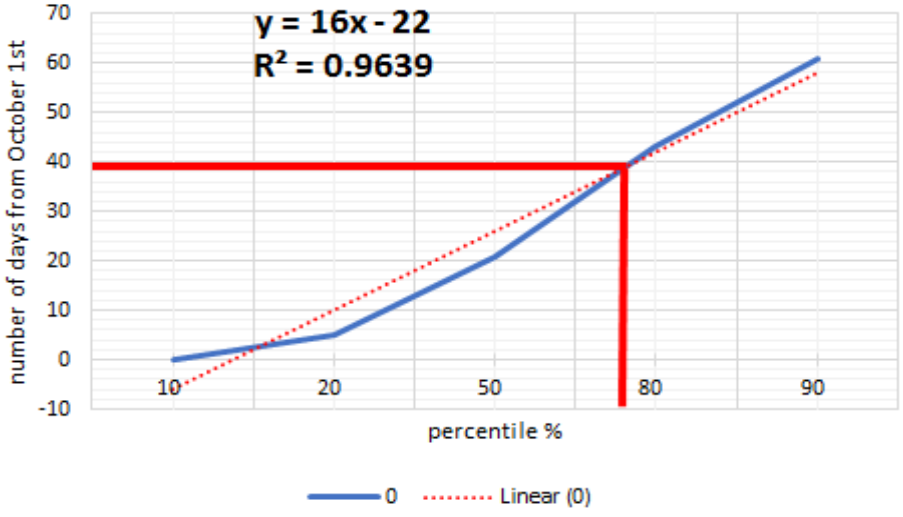

Figure 9: The day of sowing 2nd case (2 days)

The value of 75% corresponds to the 40th day from October 1st, so the sowing day is November 9th, the second week of November.

Rainfall of 20 mm in at least three days (Fig. 10):

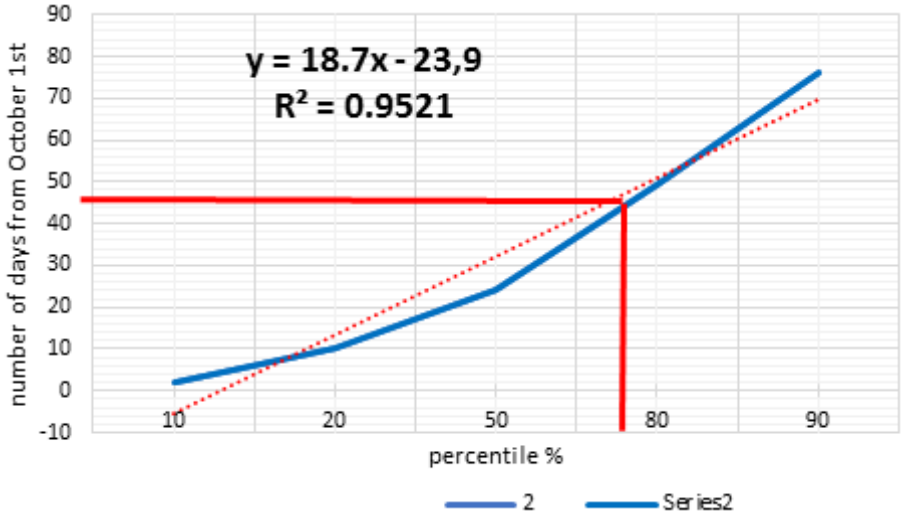

Figure 10: The day of sowing 3rd case (3 days)

The value of 75% corresponds to the 46th day from October 1st, so the sowing day is November 15th, second week of November.

Rainfall of 20 mm in four days (Fig.11)

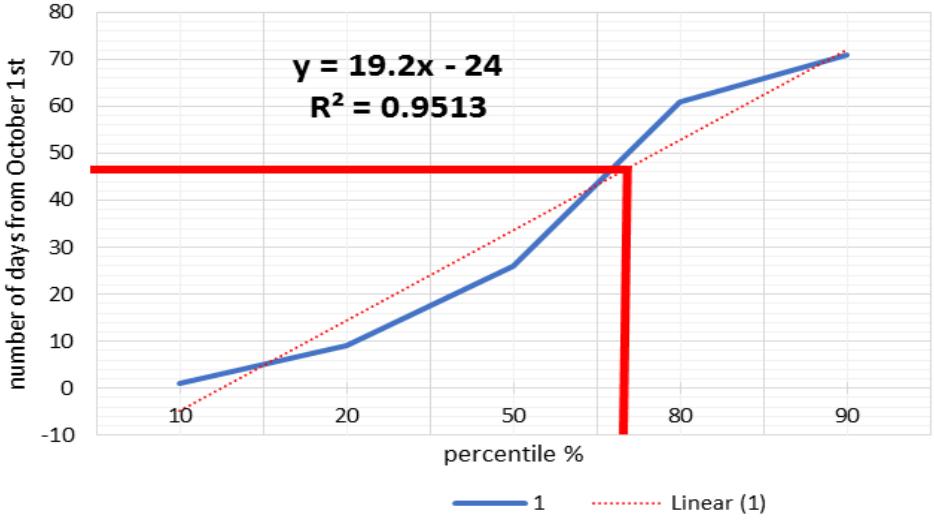

Figure 11: The day of sowing 4th case (4 days)

The value of 75% corresponds to the 56th day from October 1st, so the sowing day is November 25th, the fourth week of November.

The second scenario: with 11 days (Figs 12 to 14, and table 6)

Rainfall of 20 mm in at least one day (Fig. 12):

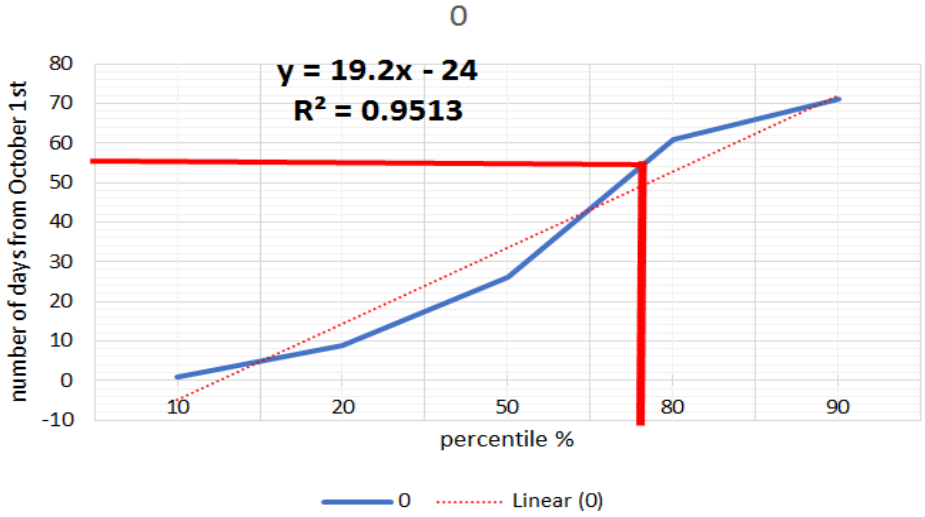

Figure 12: The day of sowing 1st case (1 day)

The value of 75% corresponds to the 55th day from October 1st, so the sowing day is November 24th, the fourth week of November. ***Note:*** Same as with 10 days.

Rainfall of 20 mm in at least two days:

Same for 11 days with at least 1 day and also 10 days with at least 1 day.

Rainfall of 20 mm in at least three days (Fig. 13):

The value of 75% corresponds to the 44th day from October 1st, so the sowing day is November 13th, second week of November.

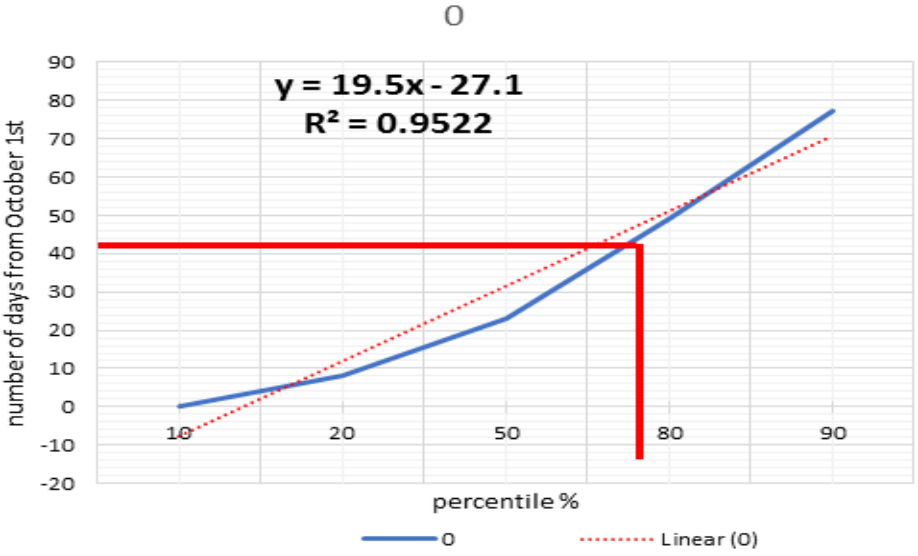

Figure 13: The day of sowing 3rd case (3 days)

Rainfall of 20 mm in four days (Fig. 14):

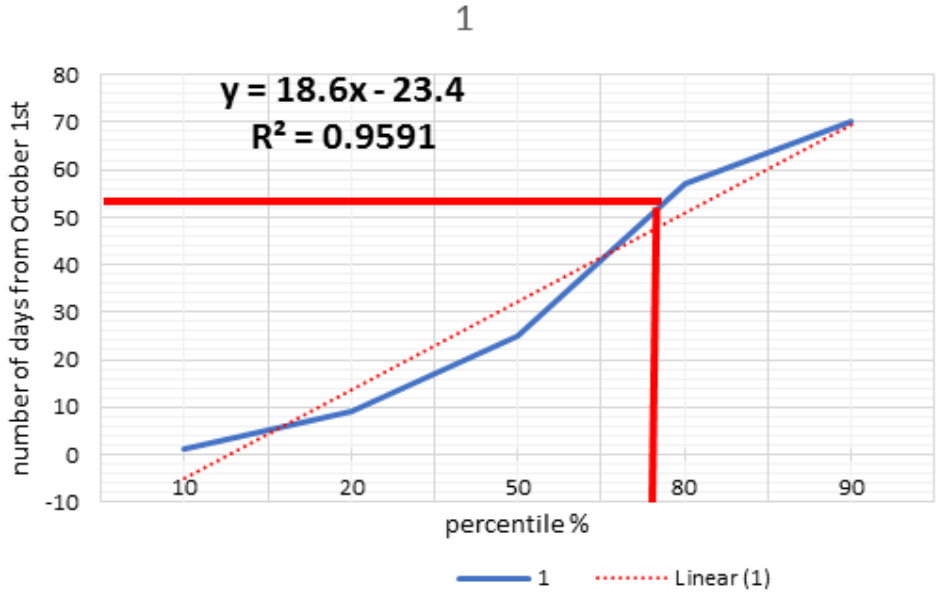

Figure 14: The day of sowing 4th case (4 days)

The value of 75% corresponds to the 53rd day from October 1st, so the sowing day is November 22nd, the second week of November.

Table 6: Summary of the results

Scenarios	**10days**			**11days**		
Numbers of days	Day. Opportunity	Correlation Coefficient	**Number of days from October**		Correlation Coefficient	Day. Opportunity
1st	24 November	**0.95**	**55**	**55**	**0.95**	24 November
2nd	9 November	**0.96**	**40**	**55**	**0.95**	24 November
3rd	15 November	**0.95**	**46**	**44**	**0.95**	13 November
4th	25 November	**0.95**	**56**	**53**	**0.96**	22 November

According to the statistical series, the best day for seeding in the first scenario is the 9th day after October (i.e., November 09). The best is the 13th day (i.e., November 13) for the second scenario. We adopt the scenario of November 9 because it has a correlation coefficient of 96%, which means the best simulation model. We are faced with an agricultural campaign that is always late, despite the

right choice to start direct seeding; using new seeding techniques and irrigation to obtain a better yield, knowing that 2/3 of the years are dry.

Finally, by choosing direct seeding, one will benefit from the following advantages:

–Production costs can be reduced by about 500 Moroccan Dirhams/Ha,

–Sowing dates can be advanced by about 9 days,

–Sowing rates can be reduced by about 30,

–Yields can be significantly improved, especially in dry years.

CONCLUSION

Since climate always involves a degree of uncertainty, climate risk management is one of the characteristics of agricultural systems and their interaction with the environment in which they operate. In the face of recurrent droughts, the best agriculture techniques adapted to this environment are necessary, allowing for water-saving and efficient use.

The results show that climate change's impact on agriculture, particularly cereal production, could be more vulnerable if measures are assumed to reduce them.

The present study contributes to the knowledge of the issues related to climate change at El Ganzra and the measures proposed using the software "InStat" to predict sowing dates regarding the first significant rains. However, considerable effort remains to be made in agro-climatic modelling to develop practical and simple decision-making tools. The agro-climatic approach confirms and completes the climate analysis that highlighted the interest in adapting to climate change by considering the precocity of crop cycles. Therefore, these two approaches converge towards the same result, which is to eliminate late planting if we want to make better use of the limited rainfall resources in this area.

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SIGNIFICANCE AND EFFICACY OF TRITERPENE SAPONIN HERBAL DRUGS WITH EXPECTORANT ACTION IN COUGH THERAPY

SUMMARY

Medicinal plants, about 10,000 species, are used in medicine to treat diseases or preserve human health because they are rich in the content of alkaloids, glycosides, resin, essential oils, etc. Phytopreparations are very important in the treatment of productive cough, both in self-medication and on the recommendation of health professionals. Medicinal preparations containing saponosides stimulate the glands to secrete bronchial secretions through a gastropulmonary reflex mechanism, which results in the production of rarer, watery mucus in the bronchial glands. They act on thick mucus as surfactants, reduce its viscosity and facilitate expectoration. Among triterpene saponosides used as expectorants, ivy leaf extract has proven therapeutic efficacy based on *in vitro* and *in vivo* studies. For other saponin expectorants (primrose root and flower, liquorice root (radix dulcis = sweet root), senega root, grindelia herb, common polypody rhizome), only the traditional application has been documented, which is confirmed by many studies. Due to the increased application, as well as the necessary confirmations of efficacy, there is a need for future research to analyze in more detail the pharmacological effects of triterpene saponins of expectorant action and their effect on the human organism.

Keywords: HMP-herbal medicinal products, cough, ivy, primrose, liquorice and senega

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INTRODUCTION

Herbal medicines (HMP - *herbal medicinal products*) are medicinal preparations that contain active substances of plant origin, they are also called phytopreparations (Applequist, 2006; Mauseth, 2016; Petrović, 2016; Heinrich *et al.*, 2018; Petrović *et al.*, 2021). They are obtained from plant organs or products that are formed in their parts (fatty oils, essential oils, resins). Medicinal properties of plants are defined by one or more pharmacologically active substances (API-*active pharmaceutical ingredient)*, a group of ingredients with similar or different chemical structure (Mauseth, 2016; Heinrich *et al.*, 2018; Watson, 2019; Popović *et al.*, 2021).

Medicinal properties can be derived from secondary metabolites, a combination of different compounds (alkaloids, tannins, phenols), which are stored only in certain parts of the plant or in the whole plant (Heinrich *et al*., 2018; Popović *et al*., 2018; Šarčević Todosijević *et al*., 2019). The compounds are specific and depend on the family, genus and species of plant, some are constantly present in plants, while some occur in response to the presence of microorganisms or physical injury (Šarčević Todosijević *et al*., 2019). Medicinal substances are extracted from herbal drugs by various pharmaceutical-technological processes and are part of traditional, but also modern medicine (Applequist, 2006; Watson, 2019).

The compounds found in plants can have a physiological effect on the organism, and for that reason their efficiency is determined by long-term tests, and the acquired knowledge is used in the production of medicinal preparations. Each phytopreparation has a certain pharmacological effect and a procedure for application (Heinrich *et al.*, 2018; Šarčević Todosijević *et al*., 2018; 2019; Popović *et al*., 2019). The effect of medicinal products containing medicinal herbs depends on the content of active pharmaceutical ingredients and combinations of medicinal herbs contained in these preparations. Compared to synthetic drugs, herbal medicines take longer to exert their effects and have weaker therapeutic effects (Petrović & Vukomanović, 2018; Kruttschnitt *et al.*, 2020; Dročić *et al*., 2020; Đorđević *et al.*, 2020).

Assessing their effectiveness is therefore difficult. However, compounds isolated from plants are very often used for the production of modern, synthetic medicinal preparations (Heinrich *et al*., 2018; Petrović & Vukomanović, 2018).

In recent years, there has been a trend of returning to traditional organic plant production and the growing popularity of their use in treatment (Popović *et al*., 2018; Stevanović *et al*., 2019; Vojnov *et al*., 2020; Ikanović & Popović, 2020; Stevanović *et al*., 2021; Popović *et al*., 2021; Lemenkova, 2021; Kwee & Maw, 2021; Petrović *et al*., 2021). Medicinal herbs can be used at home conditions in the form of teas, macerates, tinctures, powders or juices. In the pharmaceutical industry, herbal extracts are mainly tableted or made into syrups, powders, lozenges, effervescent tablets or solutions (Applequist, 2006; Watson, 2019; Petrović *et al*., 2021).

Herbal medicines are registered on the basis of clinical confirmation of efficacy, on the basis of experimental data or data obtained from the literature. In the absence of clinical research, many years of experience in traditional medicine are accepted, provided that no side effects are recorded (Petrović *et al.*, 2016).

In Europe, the trade in herbal preparations is regulated by a European directive that regulates the quality, safety and efficacy of herbal preparations, and confirmed by the Committee on Herbal Medicinal Products (HMPC) European Medicines Agency (EMA) (CPMP/QWP/2819/00).

Preparations containing herbal components must have a standard quality, which means their physical, chemical and microbiological stability, as well as a standardized composition with a high degree of purity (HSTPD, 2019; STPAC 1996; GST 2002).

Herbal drugs are rarely fresh, so dried parts of plants or whole plants are most often used to extract medicinal substances, from which various preparations are obtained by processing. Extraction of API from plants is usually done by extraction, distillation, squeezing, purification or fermentation..Powdered herbal drugs, extracts, essential oils, fatty oils, squeezed juices or processed exudates are used as semi-finished products for the production of herbal medicines. Essential oils are obtained by squeezing, distillation or extraction (Applequist, 2006; HSTPDM, 2009; CPMP/ICH/367/96).

The method of extraction of medicinal substances from plants consists of three phases (Applequist, 2006; Petrović, 2016):

1) Analysis of extracts by mass spectrometry to identify components and their relative amounts.

2) Examination of extracts in order to determine their effect on cells and their significance for a certain disease.

3) Optimization of extracts to improve their effects.

Newer sophisticated pharmaceutical-technological separation operations yield more efficient extracts with higher concentrations of active ingredients.

In recent years, the use of medicinal herbs has been on the rise as a support to official medicine and as an adjunct to therapy, and it is often used in the initial stages of some diseases. Therefore, there is an interest and need to better examine the chemical composition of plants, isolate certain compounds and analyze their effect on the human organism (Popović *et al.*, 2021; CPMP/ICH/367/96).

According to some data, over 220 pharmaceutical companies in the world, half of which are in the USA, are working to discover API from plants, define their biological role, test biosynthetic pathways and find new biotechnologies, to confirm the effectiveness of traditional drugs and medicines. For many medicines of traditional medicine, these studies have confirmed the effectiveness, and for some new indications have been discovered or new unknown compounds have been isolated (Reynolts, 1991).

In this study, a review of triterpenoid saponin herbal expectorant drugs used in cough therapy is considered, and they are considered in HMPC/EMA.

MATERIAL AND METHODS

According to the pharmacological action, Medicinal plants are divided into two groups: medicinal plants with a mild effect (mint, chamomile, linden, pomegranate, etc.) and medicinal plants with strong effects, which produce very strong poisons such as morphine, heroin, atropine, etc., whose use and handling prescribed by the law on poisonous plants. According to the mode of action on the human body, they are most often classified into several groups: a) plants that affect the work of the heart and blood vessels; b) herbs that stimulate the nervous system; c) herbs that have a calming effect on the nervous system; d) herbs for pain relief; e) plants that regulate the work of the digestive system; f) herbs that facilitate expectoration; g) herbs that accelerate wound healing. The data about plant material and its chemical analysis were described in the previous study of (Šarčević Todosijević *et al.*, 2019). All research in this study was done "at the table" based on results of numerous studies.

RESULTS AND DISCUSSION

Medicinal plants. The greatest success in agriculture will be to achieve the desired increase in production by reducing the negative environmental conditions. This can only be achieved by implementing sustainable methods and sustainable solutions in agriculture. The fact that the agricultural activities and practices are compatible with the environment and being permanent is great importance in terms of contributing to the sustainability of the ecology (Markoski *et al.*, 2018; Tugrul, 2019, Ikanović *et al.*, 2020; Markoski *et al.*, 2021). This family also includes many medicinal species. Geographical position, climatic conditions have enabled the rich biodiversity on the mountain Bjelasica in Montenegro (Balijagić *et al.*, 2021) and Serbia (Popovic *et al.*, 2021). Growing of medicinal and aromatic plants has a long tradition in agro-ecological the conditions of Europe and lead the origin from the Mediterranean region. Herbal medicine entered Western medical history around the time of Hippocrates, and herbs were used from the 5th century BCE on, to alleviate the manifestations of a particular disease. Herbs have proven potent immunomodulatory and antiviral activities. Medicinal plants have gained great popularity for beneficial applications in animals and humans. Herbs have proven potent immuno-modulatory and antiviral activities (Popović *et al*., 2021). Areas under medicinal and aromatic plants in Serbia show in Table 1.

Table 1. Areas under medicinal and aromatic plants in Serbia, 2004-2019

Parameter	Average (000 ha)	Minimum (000 ha)	Maximum (000 ha)	CV* (%)
Serbia	1.80	1.10	2.20	39.70

Source: Unstad comtrade, database 2021; * Data processing of authors

According to the data from the Serbian Chamber of Commerce, in 2012, there was 1,337 hectares under grown plants (1,419 hectares in 2011). If we add to it a portion of the areas 960 under spice plants that are listed as vegetables and

a portion of the areas on which it is produced for foreign customers, it makes a total of approximately 20,000 hectares. According to the areas of growing, the Republic of Serbia can be divided into two production regions: the lowlands (Vojvodina) and the highlands (Central Serbia). In the former region, the most commonly grown ones are: chamomile, peppermint, marigold, lemon balm, coriander, lavender, white and black mustard, valerian, fennel, parsley, basil, cumin, dill, tarragon, marshmallow, celery, thyme, sage and some other less included species. In the hilly and mountainous part of our country, there are plantations of the following: marigold, lemon balm, arnica, lavender, gentian, sage, St. John's wort and others (Filipović & Popović, 2014).

Glycyrrhiza glabra **-** Licorice or licorice root is a medicinal plant that acts on the respiratory and digestive organs, strengthens the body's defense capabilities and improves mood. Licorice has health effects including immunomodulatory, antimicrobial, antioxidative, anti-inflammatory, antidiabetic, hepatoprotextive, antiviral, anti-infective, and radical-scavenging activities.

Hedera helix - Ivy (Fig. 1a) is a plant that belongs to a type of wild vine, originating from Europe and Asia with a moderate climate. Ivy contains a chemical substance called hederin, which has a beneficial effect on the respiratory tract and relaxation of muscle mass, it has a good effect in the treatment of cellulite, calluses, burns, ulcers and some rheumatic conditions. Ivy leaf can help with polyps in the nose, problems with the spleen, inflammation of the urinary tract etc.

Primulae - Primrose (Fig. 1b) is a perennial plant which grows throughout Europe up to an altitude of 2000 m. It blooms from March to May. Medicinal parts of the plant are *radix*, flowers and leaves. The flowers are collected in May, i.e. when the plant is in bloom while the root is collected in autumn. It has a healing effect, on expectoration, thanks primarily to triterpene saponins, and with nervousness and insomnia, in gout and migraines. It has a diuretic and antirheumatic effect.

Polygalae radix - senega (Fig.2) is a species of flowering plant in the milkwort family, *Polygalaceae*. It is native to North America, where it is distributed in southern Canada and the central and eastern United States. Senega root was marketed as a treatment for pneumonia (Popović *et al.*, 2021).

a. b. https://regiuplant.ro/en/produse/ciubotica-cucului-primula-afficinalis/ c. https://stock.adobe.com/search?k=grindelia d.

Figure 1. *Hederae helicis folium (a); Primulae officinalis* (L.) *(b); Grindelia (c); Polypodium vulgare (d)*

Cough. Cough is a protective and defensive act of the respiratory system that aims to expel mucus, foreign particles and various irritants that are inhaled or formed at the site of inflammation of the mucous membranes of the larynx, trachea and large bronchi. Cough is a protective function of the body that occurs during expiration in order to remove the contents from the airways, but it can also be an indicator of the disease.

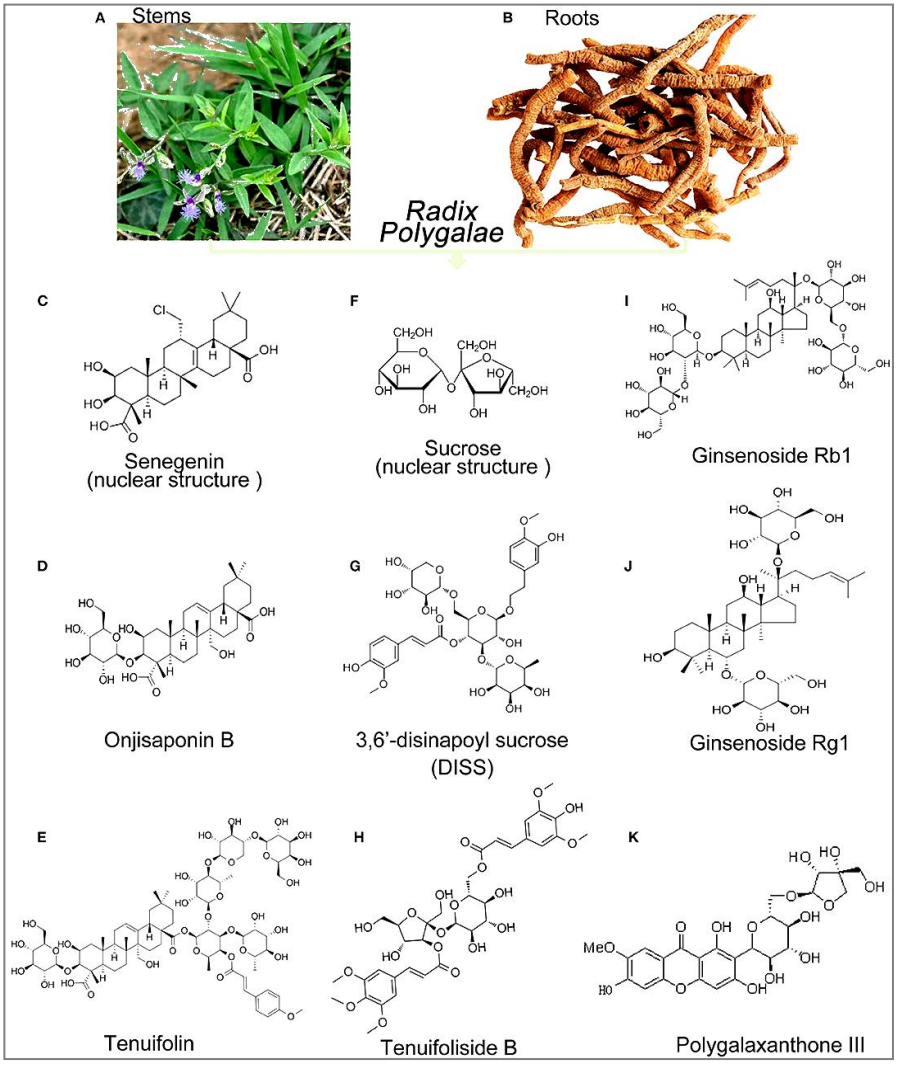

Figure 2. *Polygalae* (*Popović et al.*, 2021)

According to the duration, cough is divided into acute (lasts less than 2 weeks), subacute (lasts 2-8 weeks) and chronic cough (lasts longer than 8 weeks), and according to the pathological classification into productive and dry (irritating) cough (Kardos *et al*., 2019; Canning *et al*., 2014). The most common

causes of acute cough are upper respiratory tract diseases (viral infections), allergic rhinoconjunctivitis, pulmonary embolism, pneumothorax, as well as heartdiseases with lung congestion (Kardos *et al.*, 2019).

The most common diseases of the respiratory system - chronic obstructive pulmonary disease (COPD) and bronchial asthma, are often the causes of acute as well as chronic cough (Petrović & Vukomanović, 2019).

Subacute cough is a consequence of viral infections, but also acute bacterial sinusitis. The category of subacute cough, according to the new classification, includes viral infections caused by adenoviruses, as well as infections caused by the bacteria *Bordetella pertussis* and *Mycoplasma pneumonia*. In the etiology of chronic cough, there are diseases of the upper respiratory tract (eg. rhinitis, pharyngitis, laryngitis), non-obstructive chronic bronchitis, non-asthmatic eosinophilic bronchitis, lung cancer, etc. Other causes of chronic cough, which are not related to the respiratory system, are gastroesophageal reflux or the use of some drugs (ACE inhibitors) (Kardos *et al.*, 2019), as well as exposure to various chemical and mechanical irritants (cigarette smoke, air pollution, moisture, allergens, etc.).

The treatment of patients who have a cough depends on the etiological factors that caused it, as well as the pathogenetic basis. For these reasons, antitussives are used for irritable, nonproductive cough, while expectorants are used for productive cough (Heinrich *et al*., 2018; Petrović & Vukomanović, 2018).

Untimely and inadequate cough therapy can lead to multiple health complications, from cardiovascular and neurological to psychosocial and dermatological, which negatively affect and reduce the quality of life.

Plant expectorants. Expectorants are medicinal preparations that act on the formation, density and flow of bronchial secretions. These preparations usually contain an extract of one plant or a combination of several plant species. Their use facilitates the removal of bronchial mucus from the airways. Expectorants can be mucolytics, secretolytics or secretomotorics (Franova *et al.*, 2006; Wagner *et al*., 2015; Petrović & Vukomanović, 2018; Anheyer *et al*., 2018; Filipović *et al*., 2021).

Mucolytics change the properties of bronchial mucus, they break it down chemically, softening and reducing its viscosity. By including mucolytics in therapy, the water content in the bronchial mucus increases, which affects the increased productivity of cough.

Secretolytic preparations stimulate the mucous membrane of the stomach and respiratory tract via the cranial nerve X (*N. vagus*). The secretion of bronchial glands increases, mucus is increased, which acts to reduce its surface tension and less formation of deposits on the walls of the bronchi. The bronchial mucus is then less thick and sticky, so its flow is accelerated and the cilia make it easier to clean the airways and cough. Natural secretolytics are preparations based on mint, primrose, ipecac, ivy, liquorice, senega, thyme, eucalyptus, anise, pine and others (Filipović *et al.*, 2021). These are herbal drugs that act on the mucous membrane

of the stomach or duodenum, triggering afferent mechanisms, which causes reflex stimulation and increased secretion. They can cause vomiting if taken in higher doses because they stimulate the vomiting center.

Secretomotorics encourage the removal of mucus with better mobility of cilia, and essential oils are used for that purpose. These include essential oils of: anise, fennel, thyme, eucalyptus, white pine, juniper, larch.

Herbal cough preparations generally contain one component, but can be made from combinations of different plants (ivy, primrose, marshmallow, plantago, mint, chamomile, thyme) (Applequist, 2006; Franova *et al*., 2006; Wagner *et al.*, 2015; Mauseth, 2016; Heinrich *et al.*, 2018; Anheyer *et al.*, 2018).

Saponin expectorants. Saponosides are a type of compounds that belong to glycosides, whose structure consists of sugar (glycone) and non-sugar component (aglycone). Under the influence of hydrolytic enzymes, glycosides decompose into carbohydrates and essential oils with a pungent odor and taste. According to the aglycone component, sapogenol (or sapogenin), steroid, terpene and triterpenesaponosides are distinguished. Steroid saponosides have a hormone-like structure and are important as precursors for the synthesis of steroid hormones, while triterpenesaponosides can be toxic compounds with a weaker or stronger hemolysis ability. Saponosides are secondary metabolites of plants, which synthesize them as phytoalexins, in order to protect themselves from bacterial and fungal infections. They are dissolved in the cells of underground organs, in fruits, seeds, in some plants they are present in all parts of the plant, and in the cuticle of plants they build a waxy protective film. Due to the characteristic structure of molecules with a highly hydrophilic and lipophilic part, saponosides reduce the surface tension of heterogeneous systems, they are soluble in water, in which they build colloidal solutions. Rubbing saponin drugs in water creates foam, due to the rich foam that is formed when their solutions are mixed, they were called saponins in the 19th century (lat. *sapo*= soap). Due to their ability to act as surface active agents (SAA), they have been used in householdsfor making detergents. More recently, their properties have been used for emulsification and spraying, while their SAA property allows them to penetrate cells more easily (Applequist, 2006; Wagner *et al.*, 2015; Heinrich *et al.*, 2018).

They are safe for oral use, while parenteral use is not recommended due to their hemolytic activity. Inhalation is also contraindicated, because inhaling saponin powder triggers the sneezing reflex and can damage the airways. Saponosides damage cell membranes, leading to local irritation, and are cytotoxic at higher doses.

Most saponoside-based drugs are triterpene glycosides containing a polar glycoside (sugar) moiety and a non-polar (aglycoside) moiety, sapogenin. Sapogenins are mainly composed of pentacyclic compounds, while the glycosidic part consists of monosaccharides (D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, L-fucose) and carboxylic acids (D-glucuronic acid and D-galacturonic acid). Minor changes in the structure of molecules can affecton large differences in the activity of these compounds. They can have different

pharmacological properties, because the properties of saponosides depend on their chemical structure (Applequist, 2006; Wagner *et al.,* 2015; Heinrich *et al.,* 2018).

In recent years, the effects of triterpenesaponosides have been studied because there is growing evidence of their cytoprotective and anti-inflammatory role, as well as antitumor activity because they can induce apoptosis of tumor cells and reduce their invasiveness. However, evidences of their effectiveness were not followed by adequate explanations of the mechanism of their action (Heinrich *et al*., 2018; Petrović & Vukomanović, 2018). Saponosides react with cell membranes and their action depends on membrane structure, saponin side chains, and the nature of aglycones. When given in concentrations above 100 μm, they affect the formation of pores on the membranes, which increases the permeability (Mauseth, 2016; Heinrich *et al.,* 2018). When applied in lower concentrations, below 10 μm, they are important for physiological processes because they affect on intracellular signaling pathways. Despite a large number of studies, it is still not possible to predict the action of these compounds based on their structure. The mechanism of the expectorant action of drugs with saponosides has not yet been fully elucidated. One plant can contain several different saponosides. Some of them can be toxic (sapotoxins), and some can significantly reduce the symptoms of the disease and enable faster recovery.

Clinical studies have confirmed the effectiveness of the use of ivy herb extracts and fixed combinations of thyme herb and primrose root as expectorants in productive cough, while for a number of other herbal drugs and their preparations used in cough therapy, only traditional use has been documented (Bauer, 1973; Büechi *et al.*, 2005; Kemmerich *et al.*, 2006; Kemmerich, 2007).

Table 2. Triterpene saponosides with expectorant properties according to HMPC/EMA

Number	Species	Plant parts
1.	*Hederae helicis folium*	Ivy leaf
2.	*Primulae radix*	Primrose root
3.	*Primulae flos*	Primrose flower
4.	*Liquiritiae radix*	Sweet root
5.	*Polygalae radix*	Senega root
6.	*Grindeliae herba*	Grindelia herb
7.	*Polypodii rhizoma*	Common polypody rhizome

Herbal drugs with expectorant action belonging to triterpene saponosides, and documented in HMPC/EMA monographs are shown in Table 2, (EMA/HMPC/325716/2017;EMA/HMPC/325715/2017;EMA/HMPC/136582/2012; EMA/HMPC/571119/2010; EMA/HMPC/748220/2011).

From many herbal saponin drugs with expectorant action, only ivy leaf extract is approved by the EMA for regular therapy, because its therapeutic

efficacy has been confirmed on clinical studies in several *in vitro* and *in vivo* studies (Wagner *et al.*, 2015; Šarčević Todosijević *et al.*, 2018).

For the primrose root and flower, as well as liquorice root, senega root, grindelia herb (Fig. 1c) and common polypody rhizome, only the traditional application has been documented, and they are treated as additions to therapy. In HMPC/EMA monographs of traditional saponin expectorants, is stated that they are not used for children (liquorice root and grindelia herb are contraindicated even in adolescents under 18 years), as well as during pregnancy and lactation (ESCOP 2003, EMA/HMPC/571119/2010, EMEA/HMPC/600668/2007, Šarčević Todosijević *et al.*, 2018).

The use of grindelia (gum weed) herb (*Grindeliae herba*, *Grindelia robusta* Nutt., Asteraceae) is contraindicated for people who are hypersensitive to plants from the Asteraceae family (EMA/HMPC/748220/2011). In addition, common polypody rhizome (*Polypodii rhizoma*, *Polypodium vulgare* L. (Fig. 1d), *Polypodiaceae*) has a mild laxative effect, and is contraindicated for people with peptic ulcer (EMEA/HMPC/600668/2007).

Ivy. Ivy (*Hedera helix* L., Araliaceae) is a perennial evergreen climbing plant. It reaches a length of up to 50 meters, has leathery leaves, with green-white flower crowns collected in racemose inflorescences, the fruit is a red berry (Mauseth, 2016; Šarčević Todosijević *et al.*, 2018). Ivy is a poisonous plant, whose healing properties come from saponosides and flavonoid heterosides that have spasmolytic activity (Heinrich *et al.*, 2018).

Dried leaves (*Hederae helicis folium*) are used in therapy. They contain: triterpene saponosides 5 - 8%, hederasaponoside B and C, sesquiterpenes, flavonoids (rutin), phenolcarboxylic acids, polyacetylenes (falcarinol), phytosterols, etc. (Heinrich *et al.*, 2018; Šarčević Todosijević *et al.*, 2018).

Ivy leaf extract is very effective in diluting thick bronchial secretions and facilitates airway cleansing, so it is one of the most important and most commonly used saponin drugs with expectorant action (mucolytic). It is indicated for the treatment of uncomplicated acute bronchitis and cough therapy, which has been confirmed in *in vitro* and *in vivo* studies where its spasmolytic, bronchodilator and expectorant effects have been proven (Holzinger, 2011; Zeil *et al.*, 2014; Song *et al.*, 2015; Barnes *et al.*, 2020). The above pharmacological effects of ivy are due to saponoside, α-hederin, which is formed from hederacoside C in the body, and is considered the most important API of ivy leaves. The secretolytic effect of α-hederin is achieved through β_2-adrenergic receptors, because it increases the amount of surfactant in type II pneumocytes in the alveoli, which results in dilution of bronchial mucus and facilitation of expectoration (EMA/HMPC/325716/2017). On the other hand, the enhanced response of β_2- adrenergic receptors in the bronchi is responsible for the bronchospasmolytic and secretolytic properties of ivy leaf extract. Has been shown that α- hederin inhibit the internalization of β_2-adrenergic receptors and affect the regulation of β_2-adrenergic receptors (Büechi *et al*., 2005; EMA/HMPC/325716/2017). In addition to its effect on the respiratory system,

ivy leaf extract acts as a keratolytic and rubefacient of the skin, has antioxidant and antiviral effects, and thanks to polyacetylene (falcarinol) it also has antibacterial, antifungal, analgesic and sedative effects (Holzinger, 2011; Song *et al.*, 2015; Heinrich *et al.*, 2018; Kardos *et al.*, 2019). Unlike spasmolytic, bronchodilator and expectorant effects, these pharmacological effects have no proven clinical efficacy.

Ivy leaf should not be used in the form of herbal tea, but dry, liquid and semi-solid extracts of ivy leaf should be used in liquid and solid dosage pharmaceutical forms for *per os* application, for a maximum of 7 days. To achieve the effectiveness of the preparation as an expectorant, the preparation must contain 25 mg of saponoside in one tablet or 700 mg of saponoside in 100 ml of syrup.30% ethanol is used to prepare the extract, and the daily dose of the raw drug is 0.3-0.8 grams. Cough syrup is made from a standardized dry extract of ivy leaves.

Frequent use of ivy-based preparations can cause allergic reactions (urticaria, rash, dyspnea, anaphylactic reaction) and which is mainly due to the presence of polyacetylene falcarino (Bauer, 1973; Holzinger, 2011; EMA/HMPC/325715/2017).

Primrose. *Primrose (Primula officinalis*, *Primula veris* L., Primulaceae) is a perennial herbaceous plant with yellow flowers, the fruit is a cylindrical cocoon, the leaves are ovate, rounded at the top, wrinkled, with hairs on the reverse. *Primula veris* is widespread on the edges of mountain forests, and *Primula elatior* is characteristic of wet meadows and mountain pastures (Mauseth, 2016; EMA/HMPC/748220/2011).

In the treatment of cough, dried rhizome and root (*Primulae radix etrhizoma*) are used. They have a characteristic faint odor and a spicy and unpleasant taste. The plant blooms in early spring, so the rhizome and root are removed after the plant blooms. The rhizome is elongated, dark in color, and the roots are thin, brittle, light pink (*P. elatior*) or white (*P. veris*), about 2 mm thick (EMA/HMPC/748220/2011; Mauseth, 2016).

The drug is rich in flavonoids and saponosides, it contains 5-10% of triterpene saponosides (primula acid), aglycone protoprimulagenin A (13β, 16α, 28β-trihydroxy-oleanolicacid), protoprimula-saponin A, flavonoids (gosipetin) and others. Protoprimulagenin A (pentaglycoside) *Primula elatior* contains four sugar components: glucose, galactose, rhamnose and glucuronic acid. Protoprimula-saponin A contains protoprimulagenin A and saccharose (xylose). The most important saponin aglycones from *Primula veris* are anagaligenin, priverogenin B and priverogenin-B 22-acetate. Primula acid A is the most important saponoside of primrose, which is found in the root and flower of primrose (*Primulae flos*) (EMA/HMPC/104095/2012; EMA/HMPC/136582/2012; Heinrich *et al.*, 2018).

In addition to these components, primrose root also contains phenolic glycosides (primverin and primulaverin), which are present in both species in variable amounts (up to 2.3%) and change during drying, giving a characteristic

primrose odor. There are saponosides and flavonoids in the flower, and only saponosides in the root. Flavonoids have a proven beneficial effect on blood vessels, because they reduce their fragility and permeability, and in addition, it is important that they have a vasodilatory, anti-inflammatory and antibacterial effect. The concentration of saponosides in the flower of primrose is slightly lower than in the root. Fresh root has the most saponosides, and in the dried one the concentration of medicinal ingredients decreases by prolonging the standing time. Characteristic sugars in underground organs are: primoverose, glucose and volemitol. Underground organs do not contain quinoid compounds such as primin, which cause allergic reactions as in the aboveground parts of species of the genus *Primula.* All organs of primrose contain a lot of ascorbic acid, mostly leaves and flowers. The leaf also contains β-carotene (EMA/HMPC/104095/2012; EMA/HMPC/136582/2012; Heinrich *et al.*, 2018).

Primrose extract is mostly used due to its secretolytic and secretomotor action. It achieves expectorant effect by irritating the gastric mucosa, which also has an effect on the increased secretion of bronchial glands. Saponins, due to their ability to reduce the surface tension of bronchial mucus, spread from the pharynx to the surrounding mucosa and dilute the content that is present in the respiratory tract. This facilitates the expulsion of excess mucus, the cilia of the bronchial epithelium become more mobile, so the primrose extract is also a secretomotor. Since they show their effect by irritating the gastric mucosa, caution is necessary. In patients with gastritis or gastric ulcer, the use primrose preparations is not recommended. In addition to expectorant action, preparations containing primrose extract exhibit antibacterial, antiviral, antifungal and anti-inflammatory effects (Franova *et al.*, 2006; EMA/HMPC/104095/2012; EMA/HMPC/ 136582/2012).

Primrose extract is prepared in the form of a decoction or liquid extract. The extract of primrose flower is sometimes combined with other plants (eg mullein) in the cough preparations, and cough syrup is made from the roots.

According to the guidelines of the German Commission E, (LGCM, 2021) the daily dose of primrose flower extract is 2 - 4 grams of drug, and 2.5 - 7.5 grams of flower tincture, while the daily dose of primrose root extract is 0.5 - 1.5 grams of drug and 1.5 - 3 grams of root tincture.

The European Medicines Agency recommends as a lower limit 0.125 ‰ concentration of ethanol in the blood after a single oral dose. There is also no time limit for the use of primrose. High doses of primrose extract cause nausea, abdominal colic, vomiting, diarrhea (EMA/HMPC/104095/2012.; EMA/HMPC/136582/2012).

Liquorice. Liquorice (*Glycyrrhiza glabra* L., syn. *Liquiritia officinalis*, Fabaceae) is a plant with an upright stem and purple flowers, blooms in July and August. It has a branched root, the leaves are odd feathered, elliptical in shape, the fruit is a flat pod of brown color. It grows in dry sandy places or moderately moist meadows in southeastern Europe and the eastern and central Mediterranean (EMA/HMPC/748220/2011; Mauseth, 2016). Liquorice is one of the oldest

medicinal plants used for thousands of years in Indian, Chinese and European medicine.

Dried peeled or unpeeled parts of the roots (*Liquiritiae radix*) of cultivated plants are used as drugs. When dried, peeled and cut, the root is yellow, has a fibrous fracture, is dusted due to the starch present, with a visible cambium line. It has a sweet taste and a pleasant smell. The unpeeled root is light brown in color and has a smooth bark wrinkled lengthwise. After crushing the dried drug, a yellow powder with small starch grains, parts of the trachea with reticulate and dotted thickenings of the walls, bundles of fibers surrounded by calcium oxalate crystals and parts of the parenchyma were obtained (Mauseth, 2016; Heinrich *et al.*, 2018).

Liquorice root (*radix dulcis* = sweet root) contains a large number of chemical compounds, among which the most important are: amino acids, flavonoids, phytoestrogens, coumarins, vitamins, minerals, carbohydrates, fats, pentacyclictriterpenes and many others. It contains saponin glycoside glycyrrhizin (root 6 - 13%, extract 20 - 25%) and triterpene glycyrrhizinic acid. Glycyrrhizin is a mixture of the potassium and calcium salts of glycyrrhizinic acid. Hydrolysis of glycosides produces aglyconeglycyrrhetinic acid and two molecules of glucuronic acid. Glycyrrhetinic acid has a pronounced hemolytic effect. Liquorice root contains triterpene saponins, flavonoids (formononetin, glabren, glabridin, liquiritigenin, isoliquiritigenin), coumarins and sterols. In addition to these, it contains flavonoid glycosides (liquiritin (flavanone), isoliquiritin (chalcone), liquirithoside, ramnoliquiritin, isoflavones, isoflavanones), coumarin derivatives (herniarin, umbeliferon) and asparagine. From the flavonoid glycoside liquiritoside, glucose, rhamnose and dihydroxy-flavanon are formed by hydrolysis. Fresh root contains fatty and mineral substances, bitter ingredients, saccharose and a little mannitol. In addition to active pharmaceutical ingredients, liquorice root contains 25-30% starch, up to 10% glucose and saccharose (EMA/HMPC/571119/2010; Heinrich *et al.*, 2018).

Flavonoids and pentacyclictriterpenes have the greatest pharmacological activity. It is assumed that glycyrrhizin is responsible for the expectorant effect of liquorice extract. Saponosides locally stimulate the mucous membrane, increase salivation due to the action on the salivary glands, as well as on the bronchial glands, which affects the dilution of the mucus. Animal studies have shown central antitussive activity, which is attributed to glycyrrhizinic acid.

Also, in preclinical studies, its secretolytic action has been indicated, it has a very successful effect on the dissolution and elimination of bronchial secretion, and it is assumed that by increasing bronchial secretion, mucus is reflexively triggered and that the reflex begins in the stomach (EMA/HMPC/571119/2010).

The recommended daily dose in the treatment of cough is 5 - 15 grams of dried liquorice root (Franova *et al.*, 2006; EMA/HMPC/571119/2010).

In addition to expectorant action, liquorice root acts as a taste corrector, mild laxative, antioxidant, immunostimulant, hepatoprotective, anticoagulant and anti-inflammatory agent. Liquorice root is a very good inhibitor of COX_2 i 5-

lipoxygenase, which affects the reduced synthesis of eicosanoids and leukotrienes. It would be a great replacement for NSAIDs (nonsteroidal anti-inflammatory drugs) if there were no negative effects on the gastric mucosa. It is fifty times sweeter than saccharose, so it is used in the food industry. Liquorice ingredients are very successfully used in dermatological preparations for the treatment of hyperpigmentation.

Glycyrrhetinic acid is often an ingredient in topical preparations for the treatment of hyperpigmentation due to its strong effect on skin whitening, but it is also used in the treatment of atopic eczema, seborrhea, burns due to its anti-inflammatory properties.

Many studies indicate that long-term use of liquorice-based preparations worsens hypertension, edema, hypokalemia, and myoglobinuria occurs less frequently (due to increased concentration of myoglobin in the blood). Glycyrrhizin has a pseudoaldosterone effect because glycyrrhizinic acid blocks mineralocorticoid receptors, thereby increasing the levels of cortisol in the kidneys, which then binds to mineralocorticoid receptors.

Glycyrrhizin is metabolized in the body to glycyrrhizinic acid, which inhibits the enzyme 11-β hydroxysteroid dehydrogenase, which converts cortisol to cortisone. Higher doses of glycyrrhetinic acid affect sodium reabsorption, and due to sodium and water retention, they affect high blood pressure and hypokalemia (Franova *et al.,* 2006; EMA/HMPC/571119/2010).

Senega. *Senega* (*Polygala senega* L., Poygalaceae) is a perennial plant that grows in North America and reaches a height of about 30 cm [2].

Dried parts of the roots are used as drugs (*Polygalae radix*). The root has a characteristic unpleasant odor (due to the content of methyl salicylate), soapy taste (Heinrich *et al.,* 2018).

The chemical composition of senega root consists of saponosides 5-10%, a mixture of triterpene glycosides, senegasaponins A - D, bisdesmoside type, senegins (polygalic acid), aglycone presenegin, methyl salicylate 0.1 - 0.3%, phenolic acids, lipids, oligosaccharides, etc. (Heinrich *et al.,* 2018; ESCOP, 2003).

Senega root has a secretolytic effect because it reduces the viscosity of bronchial mucus. Senegin and polygalic acid, as well as all saponins, have an irritating effect on the gastric mucosa and reflexively increase the secretion of bronchial mucus. It is used in the treatment of productive cough in the form of encapsulated dry extract, decoction or in the form of syrup. Daily doses are 1.5-3 g of the drug, or 2.5-7.5 g of tincture (ESCOP, 2003; Wagner *et al.,* 2015; Franova *et al.,* 2018).

In addition to its expectorant action, like all other triterpene saponin expectorants, senega root is also a galactagogue (affects the increased secretion of milk in breastfeeding mothers), so it is contraindicated in lactation. It can also cause gastritis, diarrhea and emesis due to irritation of the gastric mucosa (ESCOP, 2003).

Prospective Use of Triterpene Saponin Herbal Drugs with Expectorant Action in Veterinary Medicine. In veterinary medicine, as in human medicine, there is increasing popularity of herbal drugs. However, veterinary practitioners still heavily rely on modern medicines as there is not enough scientific evidence on the benefits of herbal drugs. In recent years a few studies have provided evidence of the benefits of the usage of herbals with expectorant actions in animals (Van den Hoven, 2003; Song *et al.*, 2015). South Korean study provided evidence of expectorant and antitussive effects of *Hedera helix* (HH) and *Rhizoma coptidis (RC)* extracts mixture in mice and guinea pigs. It is found that optimal expectorant effect is dose-dependent when the mixture is prepared in a 3:1 ratio of HH and RC respectively. The benefits of the utility of *Primula* extract on the lung function in horses is studied in an Australian study (Song *et al.*, 2015). Herbal tablets Bronchipret (film-coated tablets of dried extract of *Thymus vulgaris* (160 mg/tabl) and dried *Primula veris* root extract (60 mg/tabl.)) that were used in this study had some effect on improving lung function on horses with heaves (recurrent airway obstruction syndrome), although authors report limitations of the study by using doses originaly adjusted for humans (Van den Hoven, 2003).

Ginger and rosemary oil can potentially be used in treating diseases caused by bacteria, *Escherichia coli*, *Staphylococcus aureus and Salmonella spp.* Imamović *et al.* (2021). Needless to say that this is a very promising field in veterinary as in human medicine as easiness of usage, quality of ingredients and lack of serious adverse drug effects makes herbal remedies superior to synthetic medicines.

CONCLUSIONS

Most herbal expectorants based on saponosides are triterpene glycosides, so their importance in cough therapy is dominant. In recent years, their use in the treatment of cough and as an adjunct to the treatment of respiratory infections has been increasing. Therefore, education is necessary about the rational use, as well as all interactions, side effects that may occur. In addition, there is a need to conduct more detailed analysis of the pharmacological effects of triterpene saponosides with expectorant action in order to their wider application.

ABBREVIATION

ACE inhibitors = angiotensin-converting enzyme inhibitors; SAA = surface-active agents; API = *active pharmaceutical ingredient;* EMA = European Medicines Agency; HMPC = Committee for Herbal Medicinal Products; HMP = herbal medicinal products, COPD = chronic obstructive pulmonary disease.

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MORPHOMETRIC CHARACTERIZATION OF FOUR SHEEP BREEDS REARED IN SOUTH EAST OF ALBANIA

SUMMARY

Sheep are an important livestock species in Albania. The study was conducted at the Agricultural Technology Transfer Center of Korca in South East area of Albania. This study is focused in two local sheep breeds that have the status as endangered, Shkodrane and Lara e Polisit and two imported breeds Awasi and Il de France reared in this region under the care, responsibility and management of ATTC of Korca. Morphometric measurements and indices of a 190 unrelated and randomly selected animals were analyzed. Awasi have the largest wither height (70.31), rump height (73.56), body length (117.87), chest depth (36.16). Il de France is the largest breed related to rump width (24.89) chest circumference (90.978), cannon bone circumference (8.91), body weight (55.29). Shkodrane is the smallest breed regrading to body length (93.56), chest dept (30.98), chest width (15.91), chest circumference (76.31). All breeds have rectangular body frame, with an index of body frame ranging from 153.06 to 167.87. All morphometric traits were significantly positively correlated. The analysis indicated that body weight was highly correlated with all morphometric measurements. The highest correlation was with chest circumference (0.92) and the smallest (0.65) with cannon bone circumference. The PCA of all morphometric parameters indicated that the three components accounted 90.3% of the cumulative variance. The PCA and UPGMA indicated some admixture between two Albanian sheep breeds Shkodrane and Lara e Polisit and a pure differentiation of Ile de France and Awasi. A regression analysis was performed to predict the body weight from morphometric measurements.

Keywords: body measures, correlations, regression, PCA

INTRODUCTION

Sheep are an important livestock species in Albania, especially for the local community that manage them. They are an important source of milk, meat and wool. In 2020, (Instat, 2020) (Accessed 21 April 2022) the number of sheep was 1.55 million heads, where the milked sheep were 1.17 million heads representing

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75.39 % of the total herd. Meat production in 2020 was 33 thousand tones and milk production 75 thousand litra. The Albanian local sheep breeds (autochthonous) are approximately 40% of the sheep population. The local sheep breeds have a very good adaptation to the environment and the hard conditions of their breeding (Leka, 2019)

To increase milk and meat production, over the last twenty years the interest of the farmers has moved towards the crosses of their native sheep breeds with more exotic breeds. Some public Institutions such as Agricultural Technology Transfer Center in Korça have the responsibility to organize and implement the programes for the application of crosses schemes, as well as providing advice for farmers (Jani & Kume, 2018). In Korca are found 15.79 % of the total number of sheep heads (Instat, 2020).

Genetic diversity of some local sheep breeds is estimated by the use of molecular markers like microsatellites (Hoda *et al.,* 2009) a small number of SNP (Hoda *et al*., 2011).

But there are still many local sheep populations that are not yet characterized. In the present study we are focused in morphometric traits of some local sheep populations reared in South East of Albania, that are not previously characterized. Morphological characterization of livestock breeds is very important for developing a breeding strategy in a particular production system (Deribe *et al*., 2021). Body measurements that influence positively the body weight are evaluated as indirect selection criteria in sheep breeding strategy (Çelik *et al*., 2017). The aim of this study was morphometric characterization of two local sheep breed Shkodrane and Lara e Polisit that are defined to have the endangered status and two imported sheep breeds Awasi and Il de France that are reared at ATTC in Korca and are used for the improvement of the local breeds.

MATERIAL AND METHODS

Breeds description. Two local sheep breeds "Shkodrane" and "Lara e Polisit" and two imported sheep breeds Awasi and Il de France are included in this study. These breeds are reared in the Korca region which is located in South East of Albania under the care and supervision of Agricultural Technology Transfer Center (ATTC). The responsibilities of ATTC are the implementation of conservation programme for two local sheep breeds at risk of extinction "Shkodrane" and "Lara e Polisit", and the application of breed improvement of local populations at the region through the crossbreeding with imported sheep breeds Il de France and Awasi (Leka, 2019). The center was chosen also for the availability and accessibility of the data collection.

The "Shkodrane" sheep breed is a unique autochthonous breed reared in North of Albania (Shkodra region). This breed is crossed during the second half of the last century with exotic breeds such as Cigaya and Merino, but also with another local sheep breed "Bardhoka". The results were not satisfactory. However, the crossbreed processes did not lead to the complete disappearance of the sheep populations – pure breed "Shkodrane" (Leka, 2019). The data of

CAPRA Project (2017) indicate that the population size is 700 heads with a trend the population size increment. The "Shkodrane" breeding period starts in July and ends in August. The birth weight of "Shkodrane" breed reared at ATTC in Korca is 3.16 kg for males and 2.99 kg for females. The weaning weight is 10.39kg for males and 12.64 kg for females. Average body weight in adult individuals is 50-55 kg for males and 33-35 for females. "Shkodra" sheep can be classified in the population group as "Risk of extinction" and is being strongly affected by genetic erosion (Leka, 2019)

Awasi breed was introduced for the first time in Albania from Hungary, in 1987 at the ATTC in Korca region. The Local Awassi that is raised at this region is a triple-purpose breed for meat, milk, and wool production. It has a low profile and is well adapted to the unfavorable conditions of the Middle East, where it is managed under traditionally extensive to semiextensive conditions (Xhemo & Hajno, 2013). Ile de France is also an imported breed that is reared at this center with the aim to be used for improvement of local sheep populations through the crossbreeding. According to Leka, (2019) in the ATTC the population size of Awasi and Ile de France is 90 and 75 heads respectively.

Lara e Polisit is a local breed that is raised in the central part of Albania, at Elbasani, Polis and Librazhdi region. The average body weight is 30-45 kg. The average lamb birth live weight is 1.5—2 kg. The population size at 2019 was 427 with a decreasing trend (Leka, 2019). Milk production is 40 to 45 kg per year. Animals graze in the natural pasture of the area.

Data collection. Morphometric traits of a total of 180 indivuals from the four sheep breeds (45 individuals per each breed) were recorded. The individuals, older than one year, were randomly selected from different flocks. The measurements were performed according to the (FAO, 2012) guidelines, using Lydthin's stick and flexible measuring tape (Depison *et al.,* 2021). Data were collected in January - March 2022. The linear body measurements were taken such as Wither Height (WH), Rump Height (RH), Body Length (BL), Chest Depth (CD), Chest Width (CW), Rump Width (RW), Chest Circumference (CC), Cannon Bone Circumference (CBC) and Body Weight (BW). Based on the body linear measurements the following morphological indices were calculated as described by Dauda, (2018) and Marković *et al*., (2019):

Index of Body Frame (IBF) or index of length = (body length / wither height) x100

Chest index (CI) or thorax index: (chest width / chest depth) x 100;

Index of height (IH) or index of body proportion: (wither height / rump height) x 100;

Chest depth index (CDI): (chest depth / wither height) x 100;

Index of thorax development (ITD): (chest circumference / wither height) x 100.

Dactyl thorax index (DTI): (cannon bone circumference / chest circumference) x 100;

Baron-Crevat index (BCI) or Index of conformation: (chest circumference)^2 / wither height; the higher the index – the more robust is the animal;

Relative cannon bone index (RCBI): cannon circumference / wither height x 100

Index of body weight (IBW): body weight / wither height x 100. This index indicates how compact the animal is. Meat type animals have values above 3.15. Value close to 2.75 indicates dual purpose and close to 2.60 indicates that the animals are more suitable for milk purpose.

Body index (BI) = (Body length/Chest circumference) × 100. When this measure is greater than 0.90, the animal is longiline; between 0.86 to 0.88 is medigline; and less than 0.85, it is brevigline

Proportionality (Ipr) = (Height at withers/Body length) × 100.

Area index (AI) = Height at withers × Body length

Descriptive statistics for the morphometric traits were obtained using Minitab software (Minitab, LLC, 2021). The following values were calculated: the mean, standard error (SE), coefficient of variation (CV) and minimum (Min) and maximum (Max) values. In addition, the Pearson's correlation coefficients were calculated between the morphometric traits of investigated sheep breeds and morphometric indices as well. Live weight was regressed on each of the body measurements and the best fitted regression model was assessed.

Prediction models

The full regression model of the body measurements was defined as:

$Y = a + b1X1 + b2\ X2 + b3X3 + b4X4 + b5X5 + b6X6$, where:

Y = dependent variable (body weight),

a = intercept,

b (s) = regression coefficients,

X's = independent variables (WH, RH, BL, CD, CW, RW, CC, CBC).

RESULTS AND DISCUSSION

Least square means (LSM) and standard errors (SE) for morphometric measures and body weight are shown in table 1. For all traits significant differences ($p<0.01$) were found between all breeds. The wither height (WH) ranged from 59.689 (Lara e Polisit) to 70.312 (Awasi), with an average of 64.478 at the level of whole sheep population with significant differences ($P < 0.01$) among all studied breeds. Similar order of breeds exists related to the average values of other morphometric traits such as rump height (RH), cannon bone circumference (CBN). The lowest values of the body length (BL) and chest depth (CD) are found in Shkodrane and the highest values in Awasi breed, meanwhile the lowest values for the chest width (CW), rump width (RW) and chest circumference (CC) are found also in Shkodrane and the highest values are found in Il de France. All breeds have greater rump heights than wither heights.

The Coefficient of Variation (CV) values range from 7.07 (RH) to 25.01 (BW). Coefficient of variation of BW was 2-3 times greater than other body measurements. The same order was observed also by Sabbioni *et al*., (2020) in Cornigliese sheep breed. The higher the coefficient of variation the more heterogenous in nature are the traits therefore possess more room for genetic improvement through selection (Abbaya & Dauda, 2018). The selection that exists in the morphometric traits can be exploited for the breed improvement.

Table 1. The descriptive statistics, least square means (LSM) with standard errors (SE), coefficient of variation (%), minimum and maximum values of of body measurements (cm) and body weight (kg) of four sheep breeds

Variable	Awasi	Il de France	Shkodrane	Lara e Polisit	Whole population	CV (%)	Min	Max
WH	70.32±0.50	66.67±0.41	61.25±0.37	59.69±0.35	64.47±0.38	7.84	53	79
RH	73.56±0.45	70.2±0.35	64.25±0.29	63.65±0.36	67.91±0.36	7.07	56	81
BL	117.87±0.63	104.65±0.5	93.56±0.82	98.87±0.64	103.73±0.75	9.7	81	127
CG	36.16±0.34	35.58±0.43	30.98±0.3	32.89±0.35	33.89±0.23	9.25	26	42
CW	19.09±0.25	21.23±0.23	15.92±0.22	17.12±0.19	18.33±0.19	13.58	13	25
RW	22.54±0.26	24.89±0.22	19.27±0.3	19.69±0.18	21.59±0.21	12.94	15	28
CC	86.47±0.61	90.98±0.57	76.32±0.57	76.72±0.65	82.62±0.56	9.04	68	99
CBC	7.14±0.06	8.92±0.10	6.96±0.06	6.89±0.06	7.472±0.071	12.77	6	10
BW	54.12±0.70	55.29±0.99	33.63±0.61	35.89±0.61	44.73±0.83	25.01	25	68

WH – wither height, RH – rump height, BL – body length, CD – chest depth, CW – chest width, RW – rump width, CC –chest circumference, CBC – cannon bone circumference, BW – body weight.

The morphometric indices are helpful for describing the proportions among body parts of animals. Index of Body Frame (IBF) indicate how compact the animal is (Dauda, 2018). IBF values (table 2) range from 153.062 (Shkodrane) to 167.87 (Awasi) which indicate that all breeds have rectangular body frame. Rectangular body frame have also some Pramenka sheep breeds such as Bela Krajina, Istrian Pramenka, and the Sjenicka breed (Marković *et al*., 2019).

All breeds displayed low values of chest index (CI) ranging from 52.109 (Lara e Polisit) to 59.957 (Il de France) and significantly different between breeds. These results are comparable with the values of this index found in Zeta Zuja (Marković *et al*., 2019).

ITD values range from 123.178 (Awasi) to 136.558 (Il de France). CDI values were higher than 50 in all breeds. These both indices obtained in this study indicate a good thorax development of all breeds. These breeds are reared in Korca region, which has an elevation of approximately 1400 m, therefor a high ITD indicate a better fitness and capacity of the respiratory system (Marković *et al*., 2019). The values of BCI range from 95.32 (Shkodrane) to 136.59 (Ile de France) with an average 106.3 ate the level of whole population. These values

indicate that all the breeds are not robust. The results are similar with the results found in Albanian Bardhoka breed (Hoda & Hajno, 2021). DTI also indicates thoracic development. DTI in light animals is lower than 10.5. These values range from 8.26 (Awasi) to 9.799 (Ile de France), with significant differences between populations ($p<0.01$). These values indicate that all breeds consist of light individuals and are more appropriate for milk production than for meat production (Marković *et al*., 2019). These breeds are adapted to waking longer distance, since they graze in natural pasture almost during the year and are managed under traditionally extensive to semiextensive conditions. These findings are very similar with the results obtained for some Pramenka sheep breeds by Marković *et al*., (2019). Body index (BI) values are higher than 90 in each population, indicating that the animals are longiline (Dauda, 2018).

Table 2. The least square means (LSM) with standard errors (SE) of morphometric indices of four sheep breeds

Variable	Awasi	Il de France	Shkodrane	Lara e Polisit	Whole population
IBF	167.87±1.09	157.14±0.95	153.07±1.72	165.79±1.15	160.96±0.77
CI	52.94±0.77	59.96±0.87	51.46±0.65	52.11±0.44	54.11±0.43
IH	95.58±0.27	94.97±0.34	95.34±0.4	93.8±0.25	94.92±0.17
CDI	51.48±0.47	53.41±0.63	50.58±0.36	55.09±0.43	52.63±0.27
ITD	77.06±1.04	82.94±1.42	54.9±0.93	60.09±0.9	128.35±0.61
DTI	118.33±0.97	117.49±0.74	121.23±1.32	115.23±0.7	9.04±0.06
BCI	123.18±1.08	136.59±0.9	124.71±0.94	128.57±0.93	106.29±1.09
RCBI	8.26±0.07	9.8±0.1	9.13±0.08	9±0.08	11.61±0.101
IBW	10.17±0.09	13.38±0.15	11.37±0.1	11.55±0.09	68.74±1.02
BI	34.16±0.47	31.09±0.5	30.27±0.29	26.8±0.28	125.89±0.76
Ipr	241.29±2.68	233.1±3.03	252.56±4.01	223.71±2.3	62.39±0.32
AI	410.16±2.83	391.23±2.95	406.62±4.98	390.5±2.41	6726.49±83.69

IBF – Index of body frame, CI – chest index, IH – Index of height, CDI – Chest depth index, ITD - Index of thorax development, DTI – dactyl thorax index, BCI – Baron-Crevat index, RCBI – Relative cannon bone index, IBW – Index of body weight, PI-Pelvi index, BI-Body index, Ipr-Proportionality, AI -Area index

The total phenotypic correlations among all morphometric measures for all animals is presented in the Table 3. All correlations were positive, statistically significant ($p<0.001$). The lowest value of correlation was found between CBC and BL (0.21). The strongest correlation was between WH and RH (0.957). The correlation coefficients observed within each breed (Tables S3a, b, c) were also positive, and statistically significant ($p<0.001$). All breeds individually are characterized by a high correlation between WH and RH. Awasi was characterized by a very low correlation of RW and CW (0.174). Il de France displayed the lowest correlations between CW and CBC (0.263). In Shkodrane

the lowest positive correlations were found between RW and CC (0.167) In this breed were found two negative correlations between WH and Bl, RH respectively. Meanwhile in Lara e Polisit the lowest correlations were between WH and BL (0.364). BW is highly positively correlated with all measurements. A strong positive correlation between BW and body measurements were found in different sheep breeds worldwide like in Cornigliese (Sabbioni *et al*., 2020), Arsi Bale ((Worku, 2019), Karaya ((Yilmaz *et al*., 2013) etc. Tadesse *et al*. (2014) have suggested that all the phenotypic traits that showed high positive correlation with the body weight are good estimators of body weight. The high correlations values of BW with morphometric measurements can be indirectly used for the improvement of BW (Salamon *et al* 2015). The positive correlations between body morphometric traits and body weight can be exploited for the breed improvement, since the improvement in one trait will improve also the other trait. Positive correlations values between morphometric measurements indicate a balanced physical development and adaption of the sheep breed to the environmental conditions through the process of evolution (Stojiljkovic *et al*., 2015). These finding indicate that these breeds are well adapted to the environment and climate of South East Albania that is different from the environment of their origin.

Table 3. Pearson's correlation coefficients between the morphometric traits of investigated sheep breeds

	RH	***BL***	***CD***	***CW***	***RW***	***CC***	***CBC***	***BW***
WH	0.957*	0.752*	0.683*	0.591*	0.601*	0.705*	0.379*	0.773*
RH		0.82*	0.708*	0.613*	0.647*	0.723*	0.408*	0.808*
BL			0.661*	0.55*	0.582*	0.608*	0.21*	0.756*
CD				0.624*	0.594*	0.736*	0.407*	0.749*
CW					0.915*	0.852*	0.703*	0.816*
RW						0.846*	0.739*	0.826*
CC							0.727*	0.923*
CBC								0.645*

WH – wither height, RH – rump height, BL – body length, CD – chest depth, CW – chest width, RW – rump width, CC –chest circumference, CBC – cannon bone circumference, BW – body weight. *Statistically significant correlation ($P < 0.001$)

Principal component analysis (PCA) based on the average values of all morphometric parameters for the studied sheep breeds was performed by Minitab software using varimax normalized rotation option. The analyses showed that the first two PC contributed 86 % of the total variance, while the first three factors accounted for 90.4% of the total accumulated variance, which satisfactorily explained the differences expressed in the evaluated traits. All traits that were contributing to PC1 have positive value. The most contributing were RW, CC and BW (0.346-0.375). Positive values of the first PC are found by other authors as well (Deribe *et al*., 2021; Marković *et al*., 2019). (Jolliffe, 2002) have explained that the first PC almost always has positive coefficients for all variables and

simply reflects overall ‘size’ of the individuals but the later PCs usually contrast some of the measurements with others, and can often be interpreted as defining certain aspects of ‘shape’ that are important for the breeds. The most contributing trait to PC2 was CBC (0.575). The plot of principal components is shown in figure 1.

The first component classified local sheep breeds from imported breeds. The second component clearly discriminates Awasi and Ile de France, meanwhile between Shkodrane and Lara e Polisit overlaps are observed, which indicate admixture between these Albanian local breeds.

Table 4. Eigenvalue, the proportion of variance, eigenvectors and cumulative variance of morphometric measurements and body weight in the four sheep populations

Variable	PC1	PC2	PC3
WH	0.331	-0.351	0.383
RH	0.343	-0.346	0.344
BL	0.305	-0.435	0.008
CG	0.316	-0.179	-0.81
CW	0.342	0.296	-0.07
RW	0.346	0.293	0.089
CC	0.365	0.176	-0.123
CBC	0.264	0.575	0.224
BW	0.375	0.015	-0.032
Eigenvalue	6.5276	1.2158	0.389
Proportion	0.725	0.135	0.043
Cumulative	0.725	0.86	0.904

UPGMA analysis was performed based on morphometric measurements and body indices and the dendrograme was constructed (Figure 2). The smallest distance was between Shkodrane and Lara e Polisit. The UPGMA tree dendrograme show two clusters. The first cluser include Shkodrane and Lara e Polisit. Ile de France and Awasi are grouped in the second cluster. The clusters displayed in the tree are in line also with the results of PCA. This tree structure can be related with the breeds origin. Shkodrane and Lara e Polisit are native Albanian breeds and Awasi and Ile de France are imported and reared at ATTC in Korca region for the local breed improvement reasons. Shkodrane and Lara e Polisit are raised at ATTC in Korca with the aim of conserving these local sheep breeds, therefore avoiding the mixture of breeding animals.

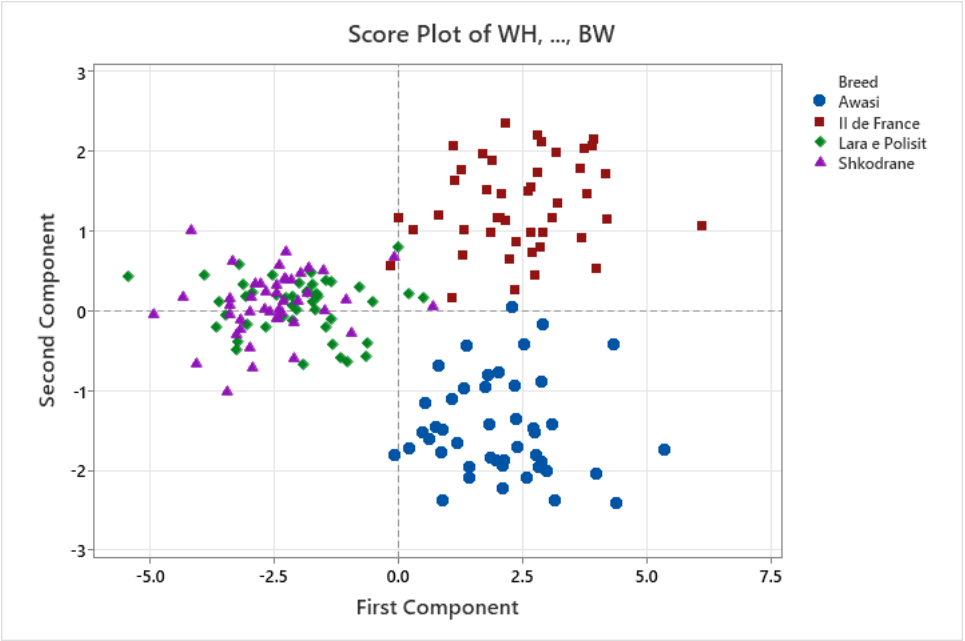

Figure 1. Scatter plot of the principal component analysis, based on morphometric measurements and body weight.

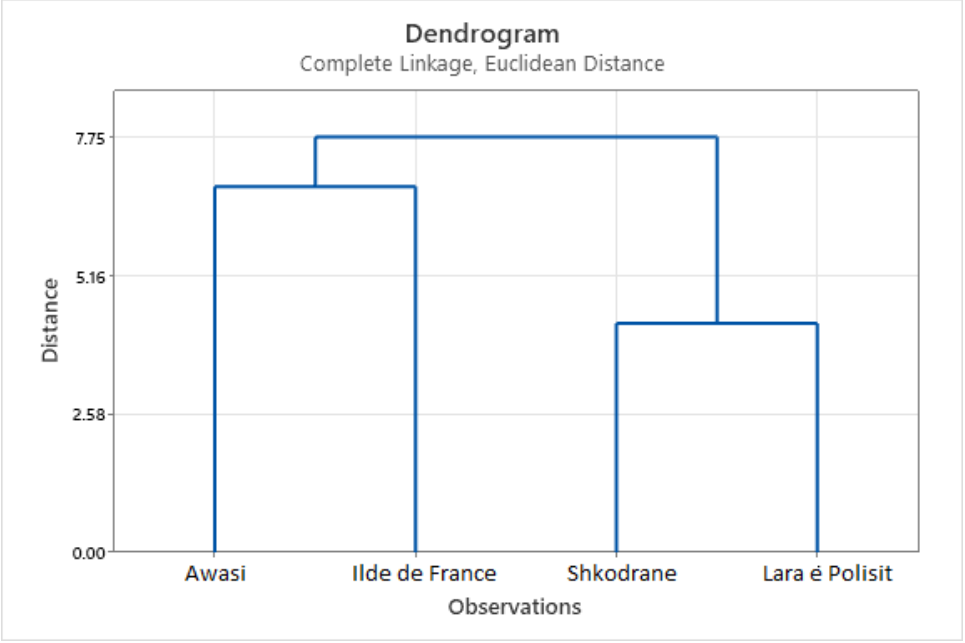

Figure 2. UPGMA tree diagram of distances sheep breeds obtained based on means for morphometric parameters and body weight

Four sheep breeds grouping based on discriminant analysis is shown in table 5. 97.8% of animals were placed in the correct group Awasi and Ile de France by the model. 93.3% of individuals were placed correctly in group Lara e Polisit. The group of Shkodrane has the lowest proportion of correct placement, 86.7%, with 39 out 45 animals were placed correctly. The results of Discriminant analysis support the PCA and UPGMa analysis, indicating admixture in the Albanian local sheep breeds, but not in the imported breeds. High level of admixture is found previously in small ruminants and is explained with the lack of herd book and with the management of these species by the farmers (Hoda *et al*., 2012).

Table 5. Breed grouping based on discriminant analysis

	True Group			
Put into Group	Awasi	Il de France	Lara e Polisit	Shkodrane
Awasi	44	0	0	0
Il de France	0	44	0	1
Lara e Polisit	1	0	42	5
Shkodrane	0	1	3	39
Total N	45	45	45	45
N correct	44	44	42	39
Proportion	0.978	0.978	0.933	0.867

There is lack of published information on prediction of BW using body measurements in Albanian sheep breeds except of Bardhoka (Hoda & Hajno, 2021). The breeds under this study are characterized here for the first time by morphological measurements. The results are shown in table 6. The analysis indicates that all predicted variables, except of WH have p-values that are less than the significance level of 0.05, which indicate that these predictors have a statistically significant effect on body weight.

Table 6. Stepwise Multiple Regression Analysis for different body linear measurements in four sheep breeds

Model	**S**	**R^2**	**R^2 (adj)**	**Mallows' Cp**	**P-value**
-69.48+1.382CC	4.32832	85.12%	85.03%	138.83	0.000
-81.88+1.101CC+0.343BL	3.35151	91.13%	91.03%	13.7	0.000
-83.36+0.9327CC+0.392BL+1.394CBC	3.25559	91.67%	91.53%	4.11	0.001
-86.93+0.886CC+0.351BL+1.426CBC+0.161WH	3.22996	91.85%	91.66%	2.36	0.053

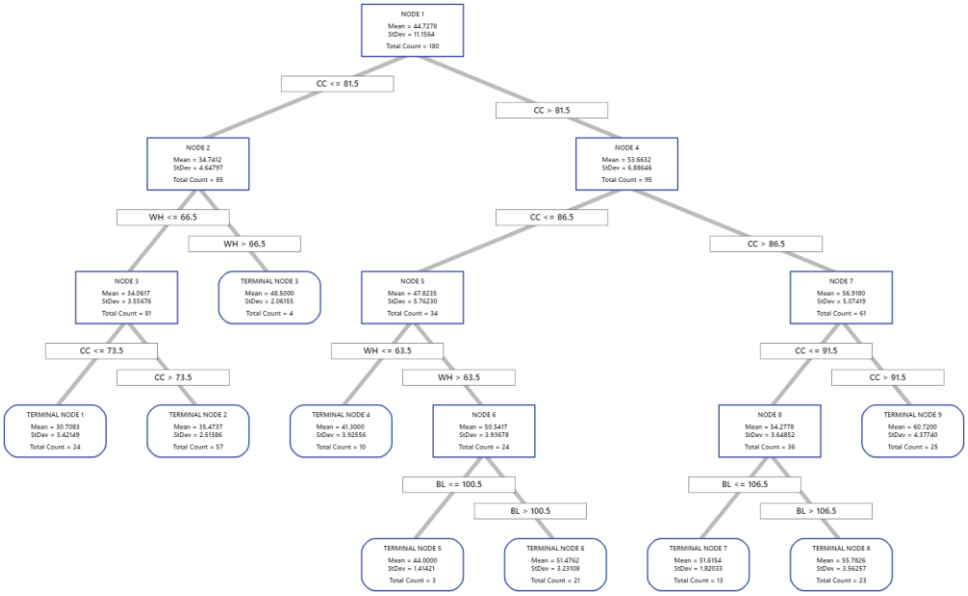

Figure 3. Regression tree diagram constructed by CART algorithm

Figure 3 shows the regression tree diagram constructed by CART algorithm in MINITAB software for the prediction of body weight from morphometric measurements. The algorithm has produced an optimal tree structure of six terminal nodes, with a Root mean squared error (RMSE) 3.207, Mean square error (MSE) 10.286, Mean absolute deviation 2.426, Mean absolute percent error (MAPE) 0.056. In figure 4, is shown the relative variable importance, which is defined as percentage of improvement related to the top predictor.

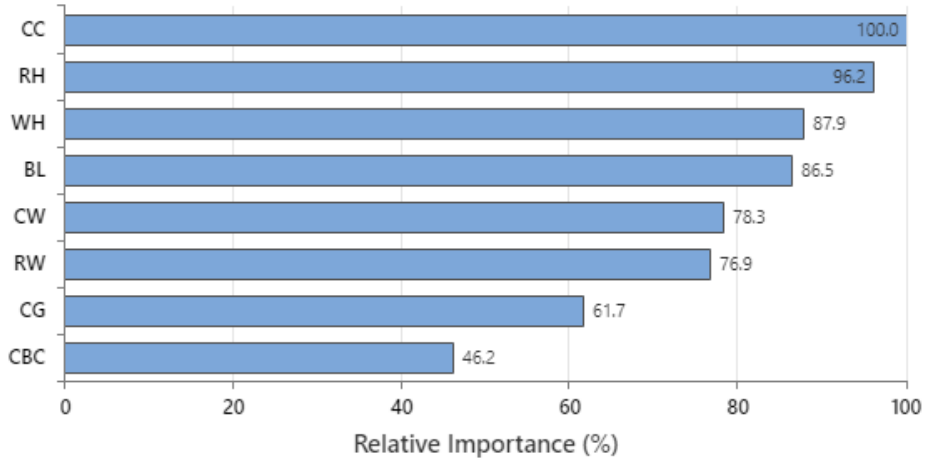

Figure 4. Relative variable importance

Assessment of body weight is important both for commercial purposes and also from the veterinary point of view where the dosages of the medicines are usually provided based on the body weight (Worku, 2019). The detection of the body measurements that are positively linked with the increment of BW in sheep breeding is vital for helping countryside economy and improving selection schemes (Faraz *et al*., 2021). This might be very helpful in the case of ATTC in Korca, whose main mission is the designing of breeding programmes for conservation of local breeds that are at risk of extinction, or the improvement programs by the crossbreeding with the imported breeds.

CONCLUSIONS

Sheep are an important livestock species of triple purpose that contribute for the development of rural economy under extensive and semiextensive conditions of Albania. The data on morphometric measurements and indices of sheep breeds raised at ATTC of Korca, in South East of Albania are used for the characterization of these breeds and the obtained results might be used in the designing of conservation and breeding programme which are the main objectives of this center. PCA, UPGMA and discriminant analysis based on body measurements could differentiate Albanian local breeds from the imported breeds. The imported sheep breeds Awasi and Ile de France are clearly distinct groups, meanwhile Shkodrane and Lara e Polisit show some level of admixture.

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VOLATILE FLAVOUR COMPOUNDS OF HERZEGOVINIAN DRY SMOKED GOAT MEAT

ABSTRACT

The volatile flavour compounds of Herzegovinian dry smoked goat meat were investigated by using headspace-solid phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS). A total of 97 volatile compounds were identified and quantified which belonged to several chemical groups: phenols (75.12%), aromatic hydrocarbons (17.47%), aldehydes (3.89%), acids (3.55%), alcohols (2.07%), ketones (1.60%), furans (0.36%), hydrocarbons (0.29%), terpenes (0.19%), esters and lactones (0.06%). The most common groups of volatile compounds in the samples of Herzegovinian dry smoked goat meat were phenols, aromatic hydrocarbons and aldehydes. Of all the identified compounds, the most common were p-cresol, creosol and 2,6-dimethoxyphenol, which were formed in the smoking process. PCA analysis showed that a positive correlation was found between the content of phenol and hydrocarbons, alcohols, ketones, esters and lactones, and terpenes, aromatic hydrocarbons and acids. Phenols as the most represented group of volatile compounds was characteristic for the leg sample (83.03%).

Keywords: Herzegovina, dry smoked goat meat, traditional product, aroma, volatile compounds

INTRODUCTION

The production of goat meat in the world, although four times smaller than the production of sheep meat, is of great importance for many countries, especially for the countries of Asia, Africa and South America. In the countries of the European Union, the production of goat meat is of significantly less importance and scope, especially in countries where dairy goat breeds are raised and where meat is a secondary product (Memiši *et al*., 2009). The importance of goat breeding has continuously declined for a long time, and in the 1990s, this branch of animal husbandry regained its importance (Žujović *et al*., 2005). That is when the imports of Boer goats, which are highly selected for meat production,

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begin. The decline in the importance of goat breeding is a consequence of the introduction of partial or complete bans on breeding goats, especially the Law on Prohibition of Goat Breeding, which was passed in 1954. The Law had a negative impact on the reduction of the number of goats, on the absence of any systematic breeding and selection work in the production, as well as on the complete extinction of already formed types or breeds (Antunović *et al*., 2012). Favorable climatic conditions, the possibility of producing cheap and good quality food, large quantities of floorcloth, smaller financial investments and the possession of certified organic land areas are very good prerequisites for the spread of organic livestock farming, including goat farming in the Mediterranean (Antunović *et al*., 2020). The importance of breeding goats in karst areas is also reflected in the cleaning of the terrain, as goats are called "terrain cleaners", which ultimately reduces the risk of fires starting and spreading. In the Republic of Croatia, in the period from 2014 to 2018, the number of goats in organic farming increased by 170.55%, and the production of goat meat by 750.0% (Antunović *et al*., 2020). The above data show the expansion of goat breeding and goat meat production in this area. Perspective of goat production in future should be more significant especially in hilly-mountainous regions, also in certain low land regions where soil quality is good only for pastures and meadows and considering production and labour potentials present on private farms it has certain advantages over other branches of livestock production (Žujović *et al*., 2005). Goat meat is a healthier alternative compared with other red meats. It has low saturated fatty acids and cholesterol (Anaeto *et al*., 2010). Goat meat has a significant role in human nutrition because it contains essential amino acids such as lysine, threonine and tryptophan (Ivanović *et al*., 2016). Based on greater nutritional value and greater unsaturated fatty acid to saturated fatty acid ratio, goat meat has the potential for improving human health and reducing obesity risk and related metabolic diseases (Mazinani & Rude, 2020). Flavor and aroma are complex attributes of goat meat. These sensory attributes are affected by species, age, fatness, and anatomical location, gender, diet and method of cooking (Webb, 2014). Aroma represents the overall perception of taste and smell. Taste is mainly associated with non-volatile compounds, such as free amino acids and small peptides that are formed at the end of the production process, while the smell is associated with the formation of volatile compounds with important aromatic properties (Marušić, 2013). The most important processes that create compounds with a direct impact on the taste and aroma of cured meat products are proteolysis and lipolysis, which involve the breakdown of proteins and lipids. Proteins and lipids constitute the major chemical components of meat and are the main substrates of action of the muscle enzyme systems. Throughout the drying stage, there exists a noticeable enzyme activity that will result in the generation of flavor precursors, such as amino acids and peptides. These precursors will contribute to the generation of flavor volatiles via the Strecker degradation system and the formation of Maillard reaction products (Flores *et al*., 1997). These changes give rise to volatile compounds such as aldehydes, carboxylic acids, alcohols, ketones, esters, sulphur and nitrogen

compounds, terpenes, alkanes and alkenes, aromatic and cyclic hydrocarbons (Jerković *et al*., 2007). Smoked products also contain phenols, which are produced by pyrolysis of lignin. In addition to the positive effect of the smoking process and the large number of useful compounds that are created by burning wood, harmful components are also created, which include Polycyclic Aromatic Hydrocarbons, i.e. PAHs. Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of chemical compounds, containing two or more fused aromatic rings made up of carbon and hydrogen atoms, known to be genotoxic agents (Ciecierska & Obiedzinski, 2007). About 660 different compounds belong to the PAH group (Đinović *et al*., 2009; Jira *et al*., 2013; Pöhlmann *et al*., 2013). These are components that have a harmful effect on the human body because certain members of this group have been found to be carcinogenic or potentially carcinogenic. The volatile compounds generated in dry-cured meat products to the final aroma depending on several parameters: concentration, odour threshold and the interaction with the food matrix (Toldrá *et al*., 2009). The production of dried goat meat is related to the wider Mediterranean area. To date, it can be found in the southern parts of France and in Corsica, Sardinia and southern Italy, and Greece (Krvavica, 2010). In Bosnia and Herzegovina, the use of young goat meat is mainly reduced to roasted young goatling (spit or brick grill). The meat of adult heads is used for the production of goat "stelja" in the areas of central and northern Bosnia, kastradina in Herzegovina and dried goat whose production is traditionally present in southeastern Bosnia (Ganić *et al*., 2013). The wider area of the municipality of Stolac is characterized by a long tradition in the production of the well-known "Herzegovina dry smoked goat meat". The peculiarity of the Mediterranean climate and plant cover, the specificity of the smell and aroma of meat, as well as the production technology, gives this traditional meat product exceptional sensory properties (Ganić *et al*., 2019; 2021). According to the Regulation of ground meat, meat preparations and meat products (Official Gazette of B&H No. 82/13), Herzegovinian dry smoked goat meat is classified as a preserved dried meat product where the moisture content must be less than 60%, water activity less than 0.93 and it must be stored at temperatures up to 15 °C. The special feature of this traditional product, along with others, is the special method of drying and smoking. Namely, drying and smoking are traditionally done on Hrgud Mountain. There are a few villages that are still inhabited on the mountain. Residents who still live in traditional mountain houses are made of stone, and their main source of income is agriculture, that is, animal husbandry. In addition, the rare inhabitants, who still live in these traditional mountain villages, are known to produce the traditional "Herzegovinian dry smoked goat meat - plaha". In the past, according to oral traditions, up to 200 plahas, which were produced in the surrounding villages, were sold weekly on the Stolac market. Nowadays, in the wider locality of the municipality of Stolac, there is only one meat processor engaged in the production of "Herzegovinian dry smoked goat meat". Its annual production is around 150 "steljas", which are mainly placed on the Sarajevo market (market places). Nowadays, with the

changes in consumer habits, goat meat is increasingly in demand on the market and is considered one of the best specialties, precisely because of its high quality and characteristic taste and smell. Accordingly, the examination of the quality of processed products and traditional goat meat products should be the subject of more research. Considering that Herzegovina dry goat meat is an autochthonous cured meat product that has not been sufficiently researched so far and is characterized by long-term traditional production, defining quality parameters of the final product would greatly contribute to the production and recognition of this product on the market. No significant research on the quality of goat meat and goat meat products has been conducted in Bosnia and Herzegovina. Therefore, the aim of this study was, for the first time, to identify volatile compounds of the aroma of Herzegovinian dry smoked goat meat. The importance of the research is reflected in the possibility of characterizing this product because some authors state that it can be performed based on isolated volatile substances. Also, the obtained research results can serve as a basis for the standardization of the production technology of this product. In the future, Herzegovina dry goat meat can represent an exceptional tourist brand that would first and foremost contribute to the development and promotion of rural areas of Bosnia and Herzegovina.

MATERIAL AND METHODS

Material. Herzegovinian dry smoked goat meat is a traditional meat product characteristic of the area of East Herzegovina, especially for the municipality of Stolac. The meat processor, which produces this meat product, owns its property on Hrgud mountain, where the animals stay all year round. Bucks intended for the production of "Herzegovinian dry smoked goat meat" are grazed exclusively on pasture, without the addition of a concentrated meal. Goats are bred in this area with a specific flora, which gives special properties to the meat. The production of this traditional meat product is characteristic only for the period from December to February. It is produced only from castrated male goats older than three years. It is known that bucks in the breeding season have a specific unpleasant smell, which originates from sex hormones. By slaughtering non-castrated animals, the smell is transferred to the meat to a significant extent, which makes it sensory unacceptable. That is why it is necessary to castrate male throats. Castration is a surgical procedure that removes the gonads (orchiectomy). For the purpose of this research, for the production of Herzegovinian dry smoked goat meat, five heads were used. Standard technology involves the following phases: bucks selection, slaughter, head and skin separation, evisceration, carcass cooling, deboning and processing of raw "stelja", salting, then drying and smoking. The bleeding of the animal is done in the traditional way so that the neck veins are cut with a knife at the level of the atlanto-occipital joint. After the bleeding ends, the head and skin are separated. The processing of raw „stelja" and boning begins by making an incision on the part of the sternum towards the spinal column and neck. Then the meat is separated from the bones by cutting the

musculature and the pelvic symphysis (*Symphysis pelvis*). The next phase involves a cut from the cranial side of the hind limbs with the separated musculature of the femoral (*Regio femoris*) and crural region (*Regio cruris*), whereby the skeleton is finally separated from the musculature. The shoulders remain within the trunk, with the shoulder blade (*Scapula*) and the upper arm bone (*Humerus*) being deboned, while the forearm bone (*Radius*) remains within the muscle tissue. To remove the shoulder blade and the upper arm bone, an incision is made on the medial side and the *capsule articularis* is opened, whereby *cavitas glenoidalis* (cavity) and *caput humeri* (head) are observed. After slaughtering the animals and primary processing of the carcasses, the meat is manually salted with coarse sea salt. Salting lasts from three to four days. The meat is then dried and smoked. The smoking procedure lasts from 15 to 20 days and is performed intermittently. The meat is dried and smoked for two days, and then the smoking is stopped. Apart from smoking and drying, all technological phases were performed in the slaughterhouse "Obradović" Stolac. Smoking and drying of meat is done in traditional stone smokehouses on the mountain Hrgud (above 1000 m). Ideal conditions for drying and smoking meat on Mountain Hrgud are the result of a characteristic climate characterized by the influence of the Mediterranean but at the same time the presence of continental currents. The relatively high altitude results very often in strong currents, which have exceptional dehydrating properties. That's why the smoking of meat takes place very briefly without intense smoking and with frequent breaks. After the technological process of production, confectioning was performed in eight parts (Figure 1).

Figure 1. Anatomical parts of Herzegovinian dry smoked goat meat (1. neck, 2. sirloin, 3. leg, 4. loin, 5. flank, 6. breast, 7. shoulder, 8. hindshank)

Methods. Volatile compounds were isolated by microextraction in the solid phase (HS-SPME) and analysed by gas chromatographic mass spectrometry (GC-MS) using a gas chromatograph 7890A (GC) associated with the mass spectrometer 5975C (MS) (Agilent Technologies, Santa Clara, CA, USA). After

defrosting, each sample was homogenized in a commercial homogenizer of food supplemented with a saturated NaCl solution and prepared according to Krvavica & Milak, (2017). After homogenization, 10 mL of the sample was quantitatively transferred to a glass vial of 20 mL in which a stirring magnet was placed, which was then sealed with a PTEF septum. For extraction, SPME was coated with DVB/Carboxen/PDMS filler (divinylbenzene/carboxy/poly-dimethylsiloxane) thickness of 50/30 μm and length 2 cm, which was conditioned for 2 min at 240 °C prior to extraction. A glass vial with a sample was placed in a water bath at 40 °C, and the sample was conditioned for 15 min at the same temperature before the fibre was inserted. The extraction was carried out for 180 min with constant stirring. The fibre was extracted from the vial upon completion of the extraction and immediately injected into the gas chromatographic injector. The injector temperature in splitless mode was 250 °C and the desorption time was 2 min. The detector temperature was set at 250 °C and the transfer line temperature at 280 °C. The helium flow rate of the carrier gas was 1.0 mL min^{-1}, and the separation of the volatile compounds was performed using DB-5ms, 30 m × 0.25 mm capillary column thickness 0.25 μm (Agilent Technologies, Santa Clara, CA, USA), in the following temperature program: initial temperature 40 °C, 10 min; 200 °C, 5 °C min^{-1}; 250 °C, 20 °C min^{-1} for 5 min. Mass spectra were obtained in Electron Ionization (EI) mode (Agilent Technologies, USA) at 70 eV at a scan rate of 1 scan per scan range of 50 to 450 m z^{-1} and the ion source temperature and mass analyser were 230 °C and 150 °C. The obtained spectra were subsequently analysed using the Enhanced ChemStation Data Analysis program, comparing the obtained mass spectra with the spectra contained in NIST 08 (US National Institute of Standards and Technology) and Wiley 8th Ed. mass spectrum databases. The identification of the volatile compounds was performed on the basis of a comparison of mass spectra, which were then confirmed by retention indexes (RI) using standard compounds for the selected volatile substances. For calibration RI, a mixture of alkane C8-C20 and pure hexane and heptane standard (Sigma-Aldrich, St. Louis, MO, USA) were analysed according to the same GC-MS program as well as the analysed samples. As the RI database, the NIST 08 mass spectrum database was used. The obtained data are shown as % of the surface area of each volatile compound in relation to the total surface of each peak. Each sample was analyzed in two replicates.

Statistical analyses. All determinations data were reported as mean ± standard deviation. For the correlation and presentation of the results multivariate data analysis was used - analysis of the basic components or PCA analysis. Statistical analyses were performed using Past software 3.15 (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

A total of 97 volatile aroma compounds of Herzegovinian dry smoked goat meat were found by gas chromatographic–mass spectrometry (GC–MS). The results of the analysis of volatile compounds are presented in Table 1. Volatile

compounds belonged to several classes of chemicals: aldehydes (8), alcohols (5), ketones (9), esters and lactones (8), hydrocarbons (2), phenols (30), aromatic hydrocarbons (24), acids (7), furans (2) and terpenes (2). Chemical groups identified in Herzegovinian dry smoked goat meat were: phenols (75.12%), aromatic hydrocarbons (17.47%), aldehydes (3.89%), acids (3.55%), alcohols (2.07%), ketones (1.60%), furans (0.36%), hydrocarbons (0.29%), terpenes (0.19%), esters and lactones (0.06%). As presented in Table 1., the most abundant compounds found in analyzed samples were phenols, aromatic hydrocarbons and aldehydes. Phenols were the most represented group of volatile aroma compounds in the tested samples. A high proportion of phenolic compounds is expected since they come from smoke and the technology of production of Herzegovinian dry smoked goat meat implies the smoking process. Also, phenolic compounds were one of the most represented groups in smoked meat products such as dalmatian dry-cured bacon (Krvavica & Milak, 2017), dalmatian dry-cured loin (Krvavica *et al*., 2018), slavonian kulen (Marušić Radovčić *et al*., 2015), dalmatian and drina dry-cured hams (Petričević *et al*., 2018). Phenols and phenolic derivated volatiles were formed primarily due to pyrolysis and oxidation of lignin, at comparatively low temperature (200–400 °C) (Marušić Radovčić *et al*., 2016). Phenolic compounds (phenols and metoxyphenols) are mainly responsible for the unique aroma and taste of smoked products. Phenols have low threshold value so their impact on the flavour of smoked products is significant (Petričević *et al*., 2018). Phenolic compounds have heavy, pungent, burnt, cresolic and smoky notes (Górska *et al*., 2017). They have an antimicrobial and antioxidative effect (Marušić Radovčić *et al*., 2019). The sample of leg part had the highest phenol content (83.03%) and the lowest was found in loin sample (68.10%). The most abundant phenols were: p-cresol (6.60-13.25%), creosol (4.73-10.71%), 2,6-dimethoxyphenol (4.17-10.49%), phenol (5.99-11.02%), 4-ethyl-2-methoxyphenol (0.00-10.00%) and 2-methylphenol (4.93-7.68%). A total of 30 phenols were identified in the tested samples, and 28 of them were found in the flank sample. The lowest number of phenols identified was in the leg sample (19), even though it had the highest content of this group of compounds. The highest p-cresol and creosol contents were found in the leg sample (13.25%; 10.71%), phenol in the sirloin sample (11.02%), and 2,6-dimethoxyphenol in the hindshank sample (10.49%). Ivanović *et al*., (2016) found three phenols in smoked goat ham phenol, 2-methylphenol and 2-methoxyphenol, while Ivanović *et al*., (2014) found only 2-methylphenol and 2-methoxyphenol in the same product. A much lower content of 4-methylphenol was found in salted and ripened goat thigh (0.08%) in a study by Paleari *et al*., (2008). In their study, Hierro *et al*., (2004) identified the following phenolic compounds in goat cecina: phenol, 2-methylphenol, 4-methylphenol, 2-methoxyphenol, and 4-methyl-2-methoxyphenol. In sheep ham in the study of Stojković *et al*., (2015) 2-methoxyphenol, 4-methyl-2-methoxyphenol and 4-ethyl-2-methoxyphenol were identified from this group. Aromatic carbohydrates were the second most common group of volatile compounds of Herzegovinian dry smoked goat meat,

which, like phenols, originate from smoke. Aromatic hydrocarbons may play an important role on the aroma of dry cured meat products due to their low odor threshold (Hazar *et al*., 2019). The most represented from this group were methoxy-phenyl-oxime (2.51-6.54%), naphthalene (3.90-5.07%) and 2-methylnaphthalene (0.00-1.97%). The highest content of aromatic hydrocarbons was found in the breast sample (20.83%) and the lowest in the leg sample (10.77%). The breast sample had the highest fat content (50.35%) and the lowest moisture content (12.61%) compared to the other examined samples in the research by Ganić *et al*., (2022). A total of 24 compounds from this group were identified in the examined samples, and in the comparison of different anatomical regions, the largest number of compounds from this group was identified in the flank sample (19). The neck sample had the highest content of naphthalene (5.07%) and 2-methylnaphthalene (1.97%), while the loin sample had a characteristic content of methoxy-phenyl-oxime (6.54%). Benzene, methylbenzene and ethylbenzene have been identified as aromatic compounds in smoked goat ham (Ivanović *et al*., 2014; 2016) and goat cecina (Hierro *et al*., 2004). Metoxy-phenyl-oxime was identified in lika lamb (0.11%) (Krvavica *et al*., 2015a), dalmatian lamb (1.81%) (Krvavica *et al*., 2015b), pag lamb (2.67%) (Krvavica *et al*., 2015c), cres lamb (3.12%) (Krvavica *et al*., 2016) and kupres lamb (2.77%) (Krvavica *et al*., 2020). The third most common group of volatile compounds of Herzegovinian dry smoked goat meat was aldehydes, which have a significant impact on the aroma of smoked products due to the low threshold of sensory detection. Straight-chain aliphatic aldehydes are typical products of lipid oxidation and could arise from the oxidation of unsaturated fatty acids (Sha *et al*., 2016). Oxidative deamination via Strecker degradation is a reaction where branched aldehydes are formed (Marušić Radovčić *et al*., 2016). The highest proportion of aldehydes was found in the neck sample (5.19%), while the smallest share in the leg sample (2.75%). In the research of Ganić *et al*., (2022) it was determined that the neck sample had the highest NaCl content (9.99%), and the leg sample had the lowest content (5.55%). In the same research, the highest value of the peroxide number was recorded in the neck sample (4.65 mmol/kg), which implies that NaCl is a promoter of lipolysis. In the neck, loin and breast samples, 6 out of 8 aldehydes were identified. The most common aldehyde in the test samples was nonanal (1.85-4.25%). In the comparison of different anatomical regions of the Herzegovinian dry smoked goat meat, the highest content of nonanal was found in the neck sample (4.25%). Nonanal contributes to flavour with sweet and fruity aroma (Marušić *et al*., 2014), and comes from the oxidation of oleic acid (García-González *et al*., 2013). Nonanal was found in smoked goat ham (Ivanović *et al*., 2014; 2016), goat cecina (Hierro *et al*., 2004) and in salted and ripened goat thigh (Paleari *et al*., 2008). Benzaldehyde and 2-furancarboxaldehyde, 5-methyl were identified in the tested samples in smaller amounts. The reaction between amino acids also produces aromatic aldehydes such as benzaldehyde, although the latter can also be formed during lipid oxidation. It contributes substantially to dry-cured meat products aroma with a

bitter almond sensory note because of its low odor threshold (García-González *et al*., 2013). The highest content of benzaldehyde was found in the sirloin sample (0.45%). Aldehyde 2-furancarboxaldehyde, 5-methyl contributes to the aroma of dried meat products with notes of almonds, burnt sugar, and caramel, and the highest content was found in the neck sample (0.27%). The acids are probably products of the oxidation of aldehydes, though they may also be originated from enzymatic lipolysis (García-González *et al*., 2013). Seven acids were identified in the samples of Herzegovinian dry smoked goat meat, and the most common were octadecanoic acid (0.08-4.43%) and n-hexadecanoic acid (0.03-5.91%). Out of a total of seven identified acids, six of them were found in the hindshank sample. The highest acid content was found in the loin sample (9.97%), and the lowest in the leg sample (0.25%). In the loin sample, n-hexadecanoic acid (5.91%) and octadecanoic acid (3.38%) were the most abundant. In addition to the above, a high acid content was recorded in the shoulder sample (9.95%), and the most abundant were oleic acid (4.35%), n-hexadecanoic acid (2.63%) and octadecanoic acid (2.40%). Straight chain alcohols come from polyunsaturated fatty acid oxidation, although low molecular weight ones most likely arise from microbial fermentation. The methyl branched alcohols can also be derived from the Strecker degradation of amino acids (Muriel *et al*., 2004). Alcohols contribute to ham flavor with herbaceous, woody and fatty notes (García-González *et al*., 2013). Alcohols have a low odour threshold, so they are crucial contributors to the aroma of meat products (Lorenzo *et al*., 2013). The largest share of alcohol was in the sirloin sample (4.36%). A total of five alcohols were identified in the mentioned sample. Other examined samples had lower alcohol content and a smaller number of identified alcohol. In the samples of Herzegovinian dry smoked goat meat, out of a total of five identified alcohols, the most represented was 2-furanmethanol (0.58-1.88%). In a comparison of different anatomical regions, the highest alcohol content was recorded in the leg sample (1.88%). The compound 2-furanmethanol, which exhibited burnt meat and vitamin-like aromas, has several suggested formation pathways such as the Maillard reaction, thermal degradation during smoking, and the deamination and dehydration of Amadori products during heating (Pham *et al*., 2008; Górska *et al*., 2017). It was also identified in polish dry-cured loin (Górska *et al*., 2017), in dalmatian dry-cured loin (0.27%) (Krvavica *et al*., 2018), in slavonian kulen (0.70%) (Marušić Radovčić *et al*., 2015), and in kazakh dry-cured beef (Sha *et al*., 2017). Ketones can be produced both lipid and by microbiological metabolism. They are considered to have a great influence on the aroma of meat and meat products, and they have a peculiar aroma, such as ethereal, butter, spicy notes or blue cheese notes (Lorenzo *et al*., 2013). Nine ketones were identified in the examined samples, and the most represented were 3-ethyl-2-hydroxy-2-cyclopenten-1-one (0.00-1.09%), 1-methylindan-2-one (0.00-0.63%) and 1-(2-furanyl) ethanone (0.11-0.56%). In the comparison of samples from different anatomical regions, the loin sample had the highest ketone content (1.97%). Six out of a total of nine ketones were identified in the leg and hindshank samples. The sirloin sample had

a characteristic content of 3-ethyl-2-hydroxy-2-cyclopenten-1-one (1.09%), the flank sample had the highest content of 2-methyl-2-cyclopenten-1-one (0.75%), and the shoulder sample had the highest content of 1-methylindan-2-one (0.63%) compared to the other tested samples. Furans, hydrocarbons, terpenes, esters and lactones were found in smaller values in the examined samples. Furans originate from smoke and Maillard reactions. Furan derivatives give aroma notes of caramel, sweet, burnt and sugar notes (Marušić Radovčić *et al*., 2015). Two compounds from this group were identified in the tested samples. Sirloin (0.67%) and breast (0.64%) samples had the highest furan content. Two compounds from the group of hydrocarbons were identified in the test samples of Herzegovinian dry smoked goat meat. In the leg sample, 1H-indene, 1-methylene (2.26%) was identified, and in the sirloin sample, 1-tetracosene (0.08%). The lower content of hydrocarbons in the examined samples is probably due to the higher content of phenols and aromatic hydrocarbons. In addition to the small content in the examined samples, their influence on the aroma is not significant because they have a high sensory detection threshold (Akköse *et al*., 2017; Lorenzo, 2014; Lorenzo i Purriños, 2013; Kaban, 2009). Two terpenes were identified in the examined samples. The neck sample had the highest terpene content (0.27%), which makes up the limonene content. Terpenes in meat products come from spices that are added during the production process, while some such as limonene may be present as a result of the diet. Namely, terpenes are normal constituents of the non-saponifying fraction of vegetable oils, which means that they originate from food and accumulate in the body of the animal (Sabio *et al*., 1998). Namely, during the production of Herzegovinian dry smoked goat meat, no spices were used, which implies that this group of compounds originates from animal nutrition.

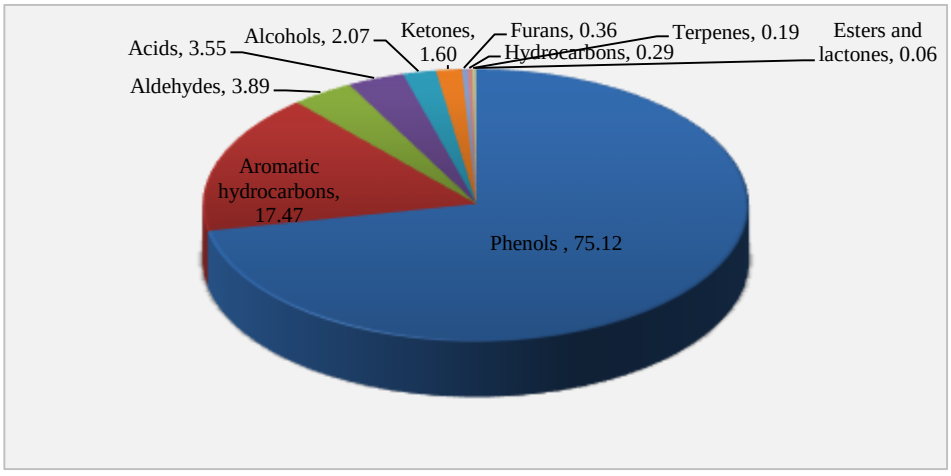

Figure 2. Volatile compounds of Herzegovinian dry smoked goat meat (% of the total peak area)

Seven esters and one lactone were identified in the examined samples. This group of volatile compounds was determined in small quantities. Ester compounds are formed by esterification of carboxylic acids and alcohols (Mateo & Zumalacárregui, 1996; Kaban, 2013). They have a very low threshold of sensory detection (Wu *et al*., 2015), and their contribution to the aroma of meat products depends on the length of the chain. Esters formed from short-chain acids have fruity notes; while esters with long-chain acids have a slight fatty odour (Pugliese i sar., 2015). Lactones are cyclic esters formed by the intermolecular reaction of the carboxyl group and the OH-group of hydroxycarboxylic acid (Velagić-Habul, 2010). The highest content of esters and lactones had the sirloin sample (0.29%). In the mentioned sample, 5 of the total 7 esters and one lactone were identified, while in the other samples, the presence of one or none of the compounds from this group was recorded.

Principal component analysis of the volatile compounds

The analysis of the main components was performed on the basis of a correlation matrix in which ten parameters were included for eight samples of Herzegovinian dry smoked goat meat. For the analysis of the main components groups of volatile compounds, namely aldehydes, alcohols, ketones, esters and lactones, hydrocarbons, phenols, aromatic hydrocarbons, acids, furans and terpenes were used as variables. The first two components that are the result of testing the above parameters of samples of Herzegovinian dry smoked goat meat contained 66.15% of the total variance, the first 42.92% and the second 23.23%.

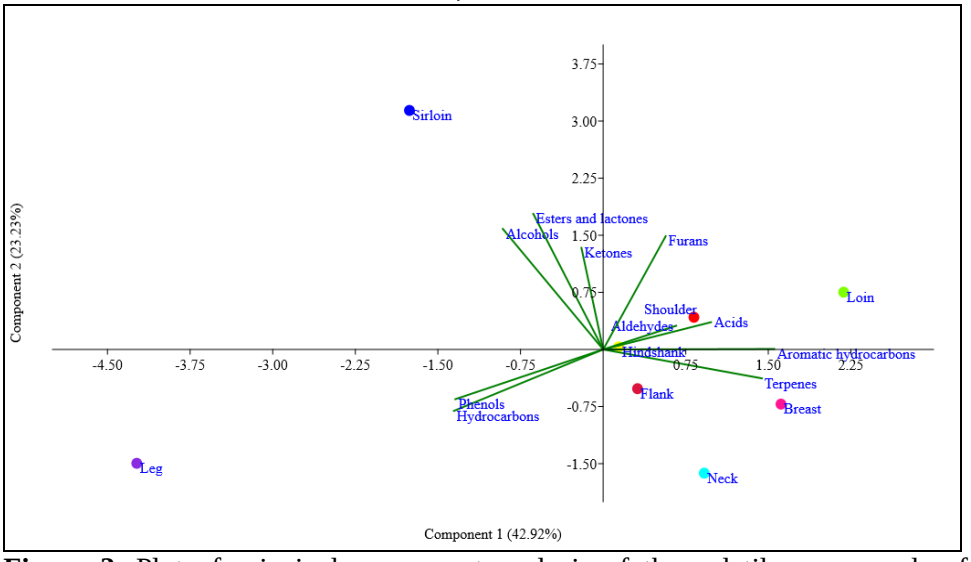

Figure 3. Plot of principal component analysis of the volatile compounds of Herzegovinian dry smoked goat meat

From the results shown in Figure 3, it can be seen that the alcohol, ketone, ester and lactone contents achieved a significant positive correlation. A positive

correlation was found between the content of terpene, aldehyde, aromatic hydrocarbons and acids. The hydrocarbon content achieved a significant positive correlation with phenol content. From the presented graph it can be seen that the aromatic hydrocarbons content was characteristic for the breast sample, while the alcohol, ester and lactone content for the sirloin sample. The phenol and hydrocarbon content were characteristic for the leg sample and the acid content for the loin sample. The hindshank sample had characteristic aldehyde content. In contrast, the content of terpene was characteristic of the neck and loin samples.

Table 1. Volatile compounds of the Herzegovinian dry smoked goat meat aroma (% of the total peak area)

RI	VOLATILE COMPOUND	Total area (%)								
		Neck	Sirloin	Leg	Loin	Flank	Breast	Shoulder	Hindshank	Identif.
ALDEHYDES		**5.19±1.20**	**4.50 ±0.38**	**2.75 ±0.58**	**4.01 ±0.24**	**3.52 ±0.75**	**4.15 ±0.36**	**2.96 ±0.32**	**4.03 ±0.32**	
795.80	Hexanal	0.07±0.10	nd	nd	nd	nd	nd	nd	nd	MS, RI
895.24	Heptanal	0.25±0.05	nd	nd	0.08 ±0.07	nd	0.21 ±0.07	nd	nd	MS, RI
955.28	Benzaldehyde	0.30±0.03	0.45 ±0.06	0.36 ±0.01	0.25 ±0.14	0.21 ±0.05	0.21 ±0.09	0.29± 0.10	0.28±0.02	MS, RI
961.55	2-Furancarboxaldehyde, 5-methyl	0.27±0.10	0.15 ±0.19	nd	0.08 ±0.07	nd	0.18 ±0.04	0.09 ±0.12	nd	MS, RI
1005.84	1H-Pyrrole-2-carboxaldehyde	nd	0.07 ±0.09	nd	0.09 ±0.11	0.11 ±0.07	0.07 ±0.08	0.13 ±0.17	nd	MS, RI
1092.04	Nonanal	4.25±0.85	3.75 ±0.70	1.85 ±0.60	3.24 ±0.06	3.01 ±0.60	3.42 ±0.32	2.45 ±0.50	3.00±0.09	MS, RI
1465.15	Benzaldehyde, 2,5-dimethoxy	nd	0.09 ±0.11	nd	nd	nd	nd	nd	nd	MS, RI
1784.51	Hexadecanal	0.05±0.07	nd	0.54 ±0.02	0.27 ±0.03	0.19 ±0.01	0.07 ±0.08	nd	0.75±0.21	MS, RI
ALCOHOLS		**1.71±0.21**	**4.36 ±3.32**	**2.2 0±0.47**	**1.49 ±0.06**	**1.95 ±0.26**	**1.04 ±0.00**	**1.60 ±0.31**	**2.24±0.24**	
865.36	2-Furanmethanol	0.99±0.14	1.56 ±0.72	1.88 ±0.42	0.94 ±0.58	1.30 ±0.07	0.58 ±0.07	1.27 ±0.74	1.79±0.10	MS, RI
941.30	2-Propanol, 1-butoxy	0.18±0.25	0.18 ±0.25	0.33 ±0.03	0.22 ±0.02	0.18 ±0.23	nd	nd	0.10±0.15	MS, RI
1099.34	Maltol	0.54±0.10	2.33 ±2.48	nd	0.33 ±0.42	0.48 ±0.09	0.46 ±0.06	0.33 ±0.43	0.35±0.48	MS, RI
1571.27	2-Tetradecanol	nd	0.21 ±0.27	nd	nd	nd	nd	nd	nd	MS, RI
1648.82	1-Tetradecanol	nd	0.09 ±0.12	nd	nd	nd	nd	nd	nd	MS, RI
KETONES		**1.14±0.31**	**1.78 ±0.08**	**1.63 ±1.05**	**1.97 ±0.09**	**1.61 ±0.43**	**1.17 ±0.50**	**1.85 ±1.47**	**1.66±1.22**	
796.02	1-Hydroxy-2-butanone	nd	nd	0.01 ±0.01	nd	nd	nd	0.01 ±0.01	0.01±0.01	MS, RI
896.16	2-Cyclopenten-1-one, 2-methyl	nd	0.22 ±0.30	0.17 ±0.05	0.15 ±0.19	0.75 ±0.47	nd	0.09 ±0.12	0.17±0.07	MS, RI
902.39	Ethanone, 1-(2-furanyl)	0.15±0.20	0.23 ±0.29	0.37 ±0.19	0.46 ±0.15	0.39 ±0.05	0.11 ±0.10	0.46 ±0.19	0.43±0.10	MS, RI
952.18	2(3H)-Furanone, dihydro-5-methyl	nd	nd	0.02 ±0.03	nd	nd	nd	nd	nd	MS, RI

RI	VOLATILE COMPOUND	Total area (%)								
		Neck	Sirloin	Leg	Loin	Flank	Breast	Shoulder	Hindshank	Identif.
1106.53	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy	0.40±0.52	1.09 ±0.22	0.47 ±0.62	nd	nd	0.68 ±0.90	0.66 ±0.89	nd	MS, RI
1188.58	1-Methylindan-2-one	0.44±0.60	nd	nd	0.51 ±0.09	0.47 ±0.01	0.38 ±0.50	0.63 ±0.49	0.43±0.60	MS, RI
1192.59	2-Hydroxy-3-propyl-2-cyclopenten-1-one	0.15±0.20	nd	nd	0.34 ±0.01	nd	nd	nd	nd	MS, RI
1286.30	1H-Inden-1-one, 2,3-dihydro-2-methyl	nd	0.25 ±0.32	0.59 ±0.24	0.51 ±0.09	nd	nd	nd	0.42±0.58	MS, RI
1907.05	Cyclopentadecanone, 2-hydroxy	nd	nd	nd	nd	nd	nd	nd	0.20±0.03	MS, RI
ESTERS and LACTONES		**0.02±0.02**	**0.29 ±0.00**	**0.05 ±0.06**	**0.02 ±0.03**	**0.00 ±0.00**	**0.00 ±0.00**	**0.11 ±0.04**	**0.00±0.00**	
808.79	Acetic acid, butyl ester	nd	0.13 ±0.18	nd	nd	nd	nd	nd	nd	MS, RI
973.18	2H-Pyran-2-one	nd	0.07 ±0.09	nd	0.02 ±0.03	nd	nd	nd	nd	MS, RI
> 2000	Octadecanoic acid, 2-propenyl ester	nd	0.01 ±0.01	nd	nd	nd	nd	nd	nd	MS, RI
> 2000	trans-13-Octadecenoic acid, methyl ester	nd	nd	nd	nd	nd	nd	0.11 ±0.03	nd	MS, RI
> 2000	Carbonic acid, decyl hexadecyl ester	0.02±0.02	nd	nd	nd	nd	nd	nd	nd	MS, RI
> 2000	Hexadecanoic acid, butyl ester	nd	0.05 ±0.06	0.05 ±0.06	nd	nd	nd	nd	nd	MS, RI
> 2000	Methyl-16-acetoxyheptadecanoate	nd	0.02 ±0.03	nd	nd	nd	nd	nd	nd	MS, RI
> 2000	Heneicosanoic acid, butyl ester	nd	0.01 ±0.01	nd	nd	nd	nd	nd	nd	MS, RI
HYDROCARBONS		**0.00±0.00**	**0.08 ±0.00**	**2.26 ±3.17**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00±0.00**	
1167.02	1H-Indene, 1-methylene	nd	nd	2.26 ±3.17	nd	nd	nd	nd	nd	MS, RI
> 2000	1-Tetracosene	nd	0.08 ±0.00	nd	nd	nd	nd	nd	nd	MS, RI
PHENOLS		**76.54±1.12**	**77.26 ±7.78**	**83.03 ±1.69**	**68.10 ±16.21**	**77.98 ±0.93**	**73.33 ±3.11**	**69.62 ±13.07**	**75.13 ±2.53**	
984.15	Phenol	7.94±0.20	11.02 ±1.75	9.71 ±0.02	5.99 ±3.71	8.90 ±0.17	6.15 ±2.23	7.60 ±4.31	6.54±0.03	MS, RI
1051.04	Phenol, 2-methyl	5.46±0.91	6.68 ±2.14	7.68 ±1.45	4.93 ±0.98	5.50 ±0.23	5.34 ±0.48	5.95 ±1.58	7.11±0.04	MS, RI
1072.20	p-Cresol/ 4-methyl phenol	11.10±0.53	12.79 ±2.05	13.25 ±1.16	9.87 ±1.90	10.70 ±0.22	11.57 ±0.13	11.24 ±1.93	6.60±9.13	MS, RI

RI	VOLATILE COMPOUND	Total area (%)								
		Neck	Sirloin	Leg	Loin	Flank	Breast	Shoulder	Hindshank	Identif.
1083.66	Phenol, 4-methoxy-3-methy	0.61±0.80	nd	nd	nd	nd	nd	nd	nd	MS, RI
1106.21	Hydrazine, (3-fluorophenyl)	nd	nd	0.57 ±0.75	0.42 ±0.58	1.12 ±0.43	nd	nd	1.00±0.08	MS, RI
1128.74	Phenol, 2-ethyl	1.43±0.09	1.10 ±0.69	1.54 ±0.05	0.96 ±0.48	1.18 ±0.07	1.03 ±0.26	1.13 ±0.48	1.42±0.06	MS, RI
1139.58	Phenol, 2,3-dimethyl-	5.18±0.49	nd	5.58 ±0.01	nd	2.04 ±2.68	3.41 ±1.88	1.97 ±2.67	2.41±3.33	MS, RI
1139.88	Phenol, 2,5-dimethy	nd	2.43 ±3.43	nd	nd	1.95 ±2.61	2.53 ±3.35	2.40 ±3.27	2.55±3.50	MS, RI
1157.19	Phenol, 4-ethyl	nd	nd	0.6 8 ±0.95	nd	1.06 ±0.05	nd	0.62 ±0.85	1.26±0.07	MS, RI
1159.58	Phenol, 3,4-dimethyl-	5.41±0.05	2.50 ±3.15	4.86 ±0.60	4.02 ±0.08	3.45 ±0.01	5.18 ±0.06	4.12 ±0.24	4.20±0.34	MS, RI
1176.13	2-Methoxy-5-methylphenol	5.41±5.20	9.25 ±2.63	10.71 ±1.19	7.56 ±1.83	8.15 ±0.64	1.15 ±0.03	1.22 ±0.17	1.40±0.02	MS, RI
1179.34	Creosol/Phenol, 2-methoxy-4-methyl	4.73±0.29	9.25 ±0.09	10.71 ±0.62	7.56 ±0.80	8.15 ±0.03	8.07 ±0.48	8.20 ±0.08	10.20±0.36	MS, RI
1198.84	Phenol, 2,4,6-trimethyl	0.22±0.29	0.43 ±0.09	1.01 ±0.63	0.58 ±0.80	0.38 ±0.03	0.78 ±0.50	0.39 ±0.08	0.74±0.36	MS, RI
1206.42	Phenol, 2-propyl	0.11±0.14	0.10 ±0.12	nd	0.11 ±0.15	0.09 ±0.11	nd	nd	nd	MS, RI
1221.51	Phenol, 2-ethyl-5-methyl	nd	0.21 ±0.29	nd	0.84 ±0.54	0.31 ±0.01	0.17 ±0.22	nd	0.19±0.36	MS, RI
1232.32	Phenol, 2-ethyl-6-methyl	1.42±1.96	1.36 ±0.31	nd	0.84 ±0.54	1.38 ±0.03	nd	0.34 ±0.04	0.92±0.75	MS, RI
1233.70	Phenol, 3-ethyl-5-methyl	1.02±0.22	0.19 ±0.24	nd	1.50 ±0.39	0.71 ±0.90	0.31 ±0.41	1.03 ±0.13	1.50±0.43	MS, RI
1238.18	Phenol, 2-ethyl-4-methyl	nd	nd	0.81 ±1.08	nd	1.17 ±0.25	0.89 ±1.18	0.75 ±1.02	nd	MS, RI
1251.47	Phenol, 2,3,5-trimethyl	1,03±0,03	nd	0,98 ±0,01	0,89 ±0,17	0,79 ±0,03	0,91 ±0,05	0,88 ±0,08	0,55±0,12	MS, RI
1251.81	4-Isopropylphenol	nd	nd	1.33 ±1.77	nd	0.50 ±0.67	nd	nd	0.95±0.99	MS, RI
1263.56	Phenol, 4-ethyl-2-methoxy	9.33±0.27	10.00 ±1.45	nd	7.30 ±1.50	7.56 ±0.62	8.43 ±0.39	8.55 ±0.73	9.43±0.25	MS, RI
1295.05	2-Methoxy-4-vinylphenol	2.80±0.06	2.46 ±0.39	nd	1.25 ±1.73	2.12 ±0.10	2.95 ±0.18	1.26 ±1.72	1.78±0.51	MS, RI
1304.78	Phenol, 5-methoxy-2,3,4-trimethyl	nd	nd	0.74 ±0.01	0.23 ±0.32	0.20 ±0.25	0.41 ±0.55	nd	0.87±0.08	MS, RI
1314.81	Phenol, 3-methyl-6-propyl	nd	0.17 ±0.23	nd	0.20 ±0.27	0.13 ±0.16	0.15 ±0.20	nd	nd	MS, RI
1332.54	Phenol, 2,6-dimethoxy	9.18±0.34	4.17 ±5.25	8.61 ±0.39	8.93 ±0.22	6.89 ±0.50	9.34 ±0.73	7.98 ±0.09	10.49±2.55	MS, RI
1340.05	Eugenol	1.14±0.07	0.99 ±0.13	1.09 ±0.06	0.96 ±0.30	0.85 ±0.06	1.41 ±0.42	0.97 ±0.04	0.53±0.74	MS, RI

RI	VOLATILE COMPOUND	Total area (%)								
		Neck	Sirloin	Leg	Loin	Flank	Breast	Shoulder	Hindshank	Identif.
1349.69	Phenol, 2-methoxy-4-propyl	2.21±0.11	0.91 ±1.15	1.97 ±0.11	2.01 ±0.56	1.62 ±0.01	1.80 ±0.01	1.86 ±0.07	1.14±1.57	MS, RI
1415.27	trans-Isoeugenol	0.77±1.01	1.24 ±0.03	1.21 ±0.04	1.15 ±0.34	1.02 ±0.10	1.28 ±0.16	1.16 ±0.06	1.35±0.66	MS, RI
1579.50	Phenol, 2,6-dimethoxy-4-(2-propenyl)	nd	nd	nd	nd	0.10 ±0.02	0.07 ±0.08	nd	nd	MS, RI
> 2000	Phenol, 4,4'-(1-methylethylidene)bis	0.04±0.05	0.02 ±0.03	nd	nd	nd	nd	nd	nd	MS, RI
AROMATIC HYDROCARBONS		**18.93±1.67**	**15.43 ±10.71**	**10.77 ±3.60**	**20.17 ±4.10**	**18.67 ±3.71**	**20.83 ±0.03**	**17.09 ±2.31**	**17.89±0.65**	
885.12	Styrene	0.04±0.05	0.05 ±0.66	nd	0.01 ±0.01	nd	0.03 ±0.04	nd	nd	MS, RI
923.90	Oxime, metoxy phenyl	5.18±3.39	5.48 ±7.51	2.98 ±3.10	6.54 ±6.54	5.84 ±1.21	6.10 ±0.36	3.56 ±0.67	2.51±0.28	MS, RI
1006.8	Benzene, 1-methoxy-4-methyl	0.05±0.07	nd	nd	nd	nd	nd	nd	nd	MS, RI
1135.75	Benzene, 1,2-dimethoxy	nd	2.12 ±2.67	nd	0.22 ±0.28	0.54 ±0.06	nd	0.21 ±0.29	0.58±0.03	MS, RI
1166.72	Naphthalene	5.07±0.23	4.59 ±0.35	4.40 ±0.31	4.14 ±0.42	3.90 ±0.12	4.11 ±0.01	4.48 ±0.48	4.74±0.06	MS, RI
1220.50	Quinoline	0.30±0.01	0.15 ±0.20	nd	0.15 ±0.21	0.21 ±0.09	nd	0.13 ±0.18	0.27±0.01	MS, RI
1235.77	Naphthalene, 1,2-dihydro-3-methyl	nd	0.13 ±0.18	nd	nd	nd	nd	nd	0.11±0.16	MS, RI
1250.79	3,5-Dimethoxytoluene	nd	nd	0.24 ±0.33	0.29 ±0.37	0.20 ±0.26	0.56 ±0.02	0.55 ±0.02	0.57±0.19	MS, RI
1322.40	1,2,4-Trimethoxybenzene	0.42±0.58	0.32 ±0.40	nd	0.67 ±0.19	0.51 ±0.12	0.81 ±0.13	0.56 ±0.15	0.88±1.09	MS, RI
1275.17	Naphthalene, 2-methyl	1.97±0.08	nd	nd	1.44 ±0.35	1.37 ±0.13	1.69 ±0.06	1.55 ±0.22	0.79±0.08	MS, RI
1291.82	Naphthalene, 1-methyl	1.29±0.06	0.75 ±0.94	0.83 ±0.08	0.54 ±0.75	0.85 ±0.06	1.06 ±0.03	0.51 ±0.70	1.05±0.03	MS, RI
1359.61	Biphenyl	1.10±0.05	0.45 ±0.63	0.87 ±0.01	0.93 ±0.17	0.74 ±0.03	0.78 ±0.25	0.89 ±0.03	0.98±0.01	MS, RI
1376.66	Naphthalene, 2-ethyl	nd	nd	nd	nd	0.28 ±0.02	0.25 ±0.12	0.29 ±0.01	nd	MS, RI
1399.82	Naphthalene, 1,5-dimethyl	0.81±0.01	nd	nd	nd	0.24 ±0.32	nd	0.58 ±0.06	nd	MS, RI
1400.61	Naphthalene, 2,3-dimethyl	1.00±0.22	nd	0.29 ±0.40	0.65 ±0.21	0.40 ±0.16	0.53 ±0.23	0.63 ±0.06	0.74±0.01	MS, RI
1404.29	Naphthalene, 2,6-dimethyl	0.57±0.79	0.68 ±0.00	nd	0.36 ±0.49	0.40 ±0.19	0.52 ±0.68	0.09 ±0.13	0.64±0,00	MS, RI
1407.55	Naphthalene, 1,7-dimethyl	nd	0.35 ±0.44	0.27 ±0.35	nd	0.26 ±0.34	nd	0.09 ±0.13	nd	MS, RI

RI	VOLATILE COMPOUND	Total area (%)								
		Neck	Sirloin	Leg	Loin	Flank	Breast	Shoulder	Hindshank	Identif.
1415.67	Naphthalene, 1,3-dimethyl	0.17±0.22	nd	0.33 ±0.43	nd	0.22 ±0.06	0.33 ±0.03	0.15 ±0.19	0.19±0.26	MS, RI
1416.88	3,5-Dimethoxy-4-hydroxytoluene	nd	nd	0.33 ±0.43	3.55 ±0.36	2.15 ±0.15	3.65 ±0.77	2.60 ±0.13	3.44±0.77	MS, RI
1435.74	Benzo[b]thiophene, 3,5-dimethyl	0.10±0.13	nd	nd	nd	nd	nd	nd	0.09±0.01	MS, RI
1444.32	Benzene, (cyclohexylmethyl)	0.11±0.14	nd	nd	nd	nd	nd	nd	nd	MS, RI
1539.13	Naphthalene, 2,3,6-trimethyl	0.18±0.25	0.21 ±0.19	nd	0.25 ±0.27	0.26 ±0.01	0.03 ±0.04	nd	0.04±0.06	MS, RI
1540.31	Naphthalene, 1,6,7-trimethyl	0.29±0.28	nd	nd	0.19 ±0.13	0.14 ±0.18	0.16 ±0.21	0.03 ±0.05	nd	MS, RI
1558.04	Fluorene	0.28±0.07	0.15 ±0.19	0.24 ±0.02	0.24 ±0.07	0.16 ±0.01	0.21 ±0.00	0.19 ±0.00	0.27±0.00	MS, RI
ACIDS		**1.00±0.64**	**0.48 ±0.42**	**0.25 ±0.10**	**9.97 ±11.83**	**1.44 ±0.68**	**4.50 ±5.68**	**9.95 ±11.99**	**0.81±0.28**	
988.86	Hexanoic acid	nd	0.28 ±0.35	nd	0.08 ±0.11	0.43 ±0.03	nd	nd	nd	MS, RI
1730.18	Tetradecanoic acid	0.18±0.00	nd	nd	0.60 ±0.62	0.10 ±0.01	0.04 ±0.03	0.35 ±0.51	0.11±0.08	MS, RI
1931.89	n-Hexadecanoic acid	0.42±0.34	0.12 ±0.02	0.14 ±0.03	5.91 ±7.2	0.37 ±0.22	0.03 ±0.04	2.63 ±2.83	0.29±0.12	MS, RI
> 2000	Oleic Acid	nd	nd	nd	nd	nd	nd	4.35 ±5.87	0.01±0.01	MS, RI
> 2000	cis-Vaccenic acid	nd	nd	nd	nd	nd	nd	0.22 ±0.31	0.01±0.01	MS, RI
> 2000	Octadecanoic acid	0.40±0.31	0.08 ±0.04	0.10 ±0.06	3.38 ±4.12	0.54 ±0.50	4.43 ±5.75	2.40 ±3.25	0.35±0.04	MS, RI
> 2000	6-Octadecenoic acid	nd	nd	nd	nd	nd	nd	nd	0.04±0.01	MS, RI
FURANS		**0.00±0.00**	**0.67 ±0.01**	**0.00 ±0.00**	**0.53 ±0.34**	**0.40 ±0.04**	**0.64 ±0.01**	**0.20 ±0.27**	**0.46±0.00**	
978.38	2(5H)-Furanone, 3-methyl	nd	0.08 ±0.01	nd	nd	0.08 ±0.01	nd	nd	nd	MS, RI
1491.37	Dibenzofuran	nd	0.59 ±0.01	nd	0.53 ±0.34	0.33 ±0.04	0.64 ±0.01	0.20 ±0.27	0.46±0.00	MS, RI
TERPENES		**0.27±0.37**	**0.13 ±0.16**	**0.07 ±0.09**	**0.24 ±0.22**	**0.24 ±0.14**	**0.20 ±0.14**	**0.22 ±0.08**	**0.19±0.02**	
1015.64	Limonene	0.27±0.37	nd	0.07 ±0.09	0.24 ±0.22	0.24 ±0.14	0.20 ±0.14	0.22 ±0.08	0.19±0.02	MS, RI
1511.01	delta,-Cadinene	nd	0.13 ±0.16	nd	nd	nd	nd	nd	nd	MS, RI

*RI-Retention index; nd-not detected

CONCLUSIONS

In all, 97 volatile aroma compounds of Herzegovinian dry smoked goat meat were found by gas chromatographic-mass spectrometry (GC-MS). Volatile compounds belonged to several classes of chemicals: aldehydes (8), alcohols (5),

ketones (9), esters and lactones (8), hydrocarbons (2), phenols (30), aromatic hydrocarbons (24), acids (7), furans (2) and terpenes (2). The most common group of volatile compounds were phenols (75.12%) derived from smoke. Of all the identified volatile compounds in the examined samples, the most abundant was p-cresol, which was determined in the range from 6.60% (hindshank) to 13.25% (leg). After phenol, the most represented groups were aromatic hydrocarbons and aldehydes. In the comparison of samples according to the anatomical region, the largest number of identified compounds was found in the flank sample (64). The leg sample had the highest content of phenols and hydrocarbons, as well as the lowest content of aldehydes, acids and aromatic hydrocarbons compared to the other tested samples. Also, the specified sample had the lowest number of identified compounds compared to other anatomical parts (45). The alcohol, furan, ester and lactone content was characteristic of the sirloin sample. The neck sample had the highest content of aldehydes and terpenes, the loin sample of ketones and acids, while the breast sample had the highest content of aromatic hydrocarbons. The results of research of volatile aroma compounds indicate that the aroma profile of Herzegovinian dry smoked goat meat is specific. The aroma of this product is the result of the specific characteristics of the meat, climatic factors, the plant material that the animals are fed with and the production technology. Future research should include the analysis of the flora of pastures and meadows to identify potential biomarkers of Herzegovinian dry smoked goat meat.

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ANTHROPOMETRIC DATA IMPORTANCE IN FURNITURE DESIGN AND MARKETING

SUMMARY

Furniture design and marketing needs to reflect the increasing anthropometric data of population in industrialised countries. With beds and mattresses, the need to accommodate users with higher than standard weight and height is even more apparent as they directly affect quality of sleep and life. In Slovakia, the weight of approximately 46,000 men with severe obesity is more than 110 kg, however the European standards only assess the strength, durability, and safety beds based on use by persons weighing up to 110 kgs. The aim of the research presented in this article was to determine whether it is possible to find and acquire an appropriate mattress for overweight and/or extra tall Slovak consumers. We examined the product portfolios of 30 mattress retailers in Slovakia to determine if they reflect the needs of the current population and contain suitable products for heavier and taller people. The method of content analysis was used, with data collected, coded, and statistically processed in July 2022. The results revealed that 87% of Slovak retailers offer mattresses for heavier people above 110 kg and 30% of retailers for extra tall consumers. Detailed results reveal the options for people with weights up to 200 kg and evaluate the comfort of finding an appropriate product for such consumers.

Keywords: anthropometric data, furniture marketing, mattress portfolio, mattresses for overweight people, mattresses for extra tall people

INTRODUCTION

The population's anthropometric features are changing. The average height and weight of both women and men are increasing over time (Sedmák and

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Hitka, 2004). Such trends have been observed and confirmed in multiple countries. In fact, over the past 1.5 centuries, the average height of people in industrialised nations has increased by approximately 10 cm (Scientific American, 1988). Hauspie *et al.* (1997) identified increases of 30 mm/decade in some countries of Southern and Eastern Europe over the last decades of the 20th century. Today's taller stature has been attributed mostly to improved socioeconomic conditions (Styne and McHenry, 1993).

The average weight of individuals is on the rise as well. Researchers are attributing the increasing percentage of overweight people to lifestyle, eating behaviour, and stress (Hruby and Hu, 2015; Sominsky and Spencer, 2014). Since 1975, worldwide obesity has almost tripled (World Health Organization, 2021). In 2017, nearly a third of the world's population was overweight or obese, with the number of obese people doubling since 1980 (Fox, 2017). In eight countries (U.S., Egypt, Turkey, Chile, Argentina, Mexico, UK, and Iraq), the female age-standardised obesity has climbed over 30% (World Economic Forum, 2016). In Slovakia, the situation is similar to other developed countries. Following the BMI data, 400,000 men in Slovakia suffered from obesity and 90,000 suffered from severe obesity in 2017 and the weight of approximately 46,000 men with severe obesity was more than 110 kg (Réh *et al.*, 2019).

Such increases in height and weight need to be reflected in furniture design (Langová *et al.*, 2019; Mokdad and Al-Ansari, 2009). Furniture needs to be made bigger and more durable. In recent years, there has been intensive research on secular population trends in Slovakia. At the same time, current standards for the dimensions and strength characteristics of seating and bed furniture are being analysed (Hitka *et al*., 2018). Furthermore, material needs to be addressed in relation to strength characteristics (Pedzik *et al*., 2020, Kwidziński *et al.*, 2021, Seng Hua *et al.*, 2022, Vilkovska *et al.*, 2018, Gejdos and Potkany, 2017). Requirements for the sizes and load-bearing capacity of furniture determined for the current population exceed the European standardised sizes. The valid European standard EN 12520:2015 assesses the strength, durability, and safety of domestic seating furniture e.g. chairs or sofas, based on use by persons weighing up to 110 kg (Furnitest, 2022). Bed testing according to EN 1725:1998 is also performed based on the nominal weight of 110 kg (European Standards, 2022). Despite the outdated standardisation beds and mattresses need to accommodate taller and heavier people than ever before.

This article focuses on the marketing perspective of this problem. It has been determined that overweight users are not taken into consideration in the process of designing and making furniture construction in Slovakia (Réh *et al.*, 2019). With beds and mattresses, the implications are broader than pure furniture durability as they affect sleep quality and subsequently quality of life (Zhang *et al.*, 2021). Therefore, the aim of the research presented in this article was to determine whether for such Slovak consumers it is possible to easily find and acquire an appropriate mattress. We examined the product portfolios of mattress retailers in Slovakia to determine if they reflect the needs of the current

population and contain suitable products for taller and heavier people. We also looked at how the information regarding mattress length and weight is presented and whether it is possible and easy to find and purchase such mattresses online.

MATERIAL AND METHODS

To assist in achieving the main objective of the paper, these research questions were defined:

RQ1: Do Slovak retailers offer mattresses for heavier people above 110 kgs?
RQ2: Do Slovak retailers offer mattresses for extra tall people?
RQ3: How easy/difficult is it for Slovak consumers to find such products online?

To provide answers to the research questions, an empirical study has been conducted using the content analysis method. Websites of 30 mattress retailers operating in Slovakia were analysed between 05 and 19 July 2022. The most prominent retailers were included in the research sample and were identified by searching in Google.sk for terms such as 'mattress', 'mattresses' or 'mattress retailer' in their Slovak language equivalent. The results on the first 5 pages were scanned and analysed and a list of online mattress retailers was created, containing the brand name and website address (URL).

By analysing the online content on these websites, certain parameters and content were observed and marked into a respective category in a spreadsheet. These parameters included: i) Menu – weight (are there certain weight categories presented in the mattress menu); ii) Menu – length (are there certain length options/categories presented in the mattress menu); iii) Firmness filter (is there an option to filter out mattresses based on their level of firmness); iv) Weight filter (is there an option to filter out mattresses based on the user weight); Length filter (is there an option to filter out mattresses based on their length); v) Weight information (does the website offer information on the maximum weight limit for the mattresses sold); iv) Portfolio details (does the website sell mattresses with higher weight limits above 110 kg and larger mattresses with more than 200 cm length).

All authors participated in defining the parameters and their thresholds. The answers were coded into the spreadsheet by the first author and checked/verified by the second author. The data was processed using descriptive statistics. Patterns were identified and the results were used to provide answers to the defined research questions.

RESULTS AND DISCUSSION

For people looking to buy a mattress in Slovakia, there is plethora of options available online. There are dozens of retailers offering mattresses from local and foreign manufacturers. For a consumer of a standard height and weight, it is very easy to order and select a mattress. However, how does the situation look like if a person of non-standard height and weight needs to purchase a mattress? Are such products available?

When it comes to selecting a mattress for a person with higher than the standard weight of 110 kg, it is of utmost importance that the retailers display the information on maximum recommended weight for each of their mattresses. Otherwise, their customers won't be able to determine which mattress suits their weight. Out of the 30 retailers, 24 (80%) displayed the information about the recommended user weight by each mattress. One retailer had this information shown by some mattresses only. 17% of retailers did not display any information about the appropriateness of their products for users of different weights.

Next, we evaluated the choice of mattresses for people of higher weight. We examined the portfolio of each retailer and detected whether they have mattresses on offer that would suit a person with the weight of 120 kg, 130 kg, 140 kg and so on. The results are displayed in Figure 1. As it can be seen, 87% of retailers offer at least one option for users weighing 120 kg. Customers weighing 130 kg still have a good choice in terms of point of sale, with 83% of retailers having a mattress suiting their weight. Mattresses for customers with 140 kg can be found at 73% of retailers and for 150 kg customers at 67% of retailers. However, when it comes to even heavier people, the offer gets significantly reduced with only 30% retailers offering a product for users weighing 160 kg. The highest weight recommended for a mattress user was 200 kg and such products are on offer by 10% of retailers.

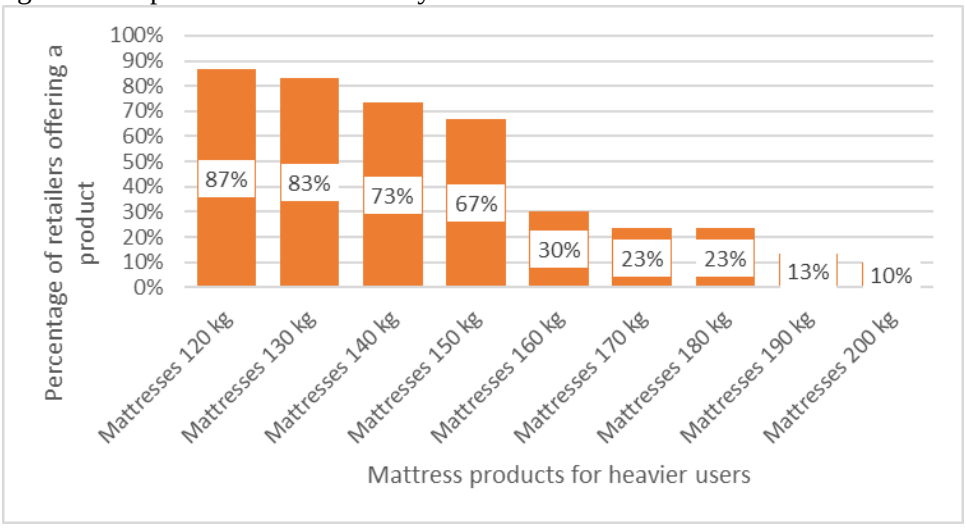

Figure 1. Availability of mattresses for heavier users

Most retailers (70%) only offer mattresses of standard length 200 cm. For taller people this does not need to be sufficient. Nine retailers (30%) have a mattress for extra tall people on offer. In case of one retailer (3.33%) the length was set to 210 cm, by three retailers (10%) this was 220 cm, and five retailers (16.7%) offered mattresses of custom lengths (and width) that can be manufactured as per the customer requirements. Results are depicted in Figure 2.

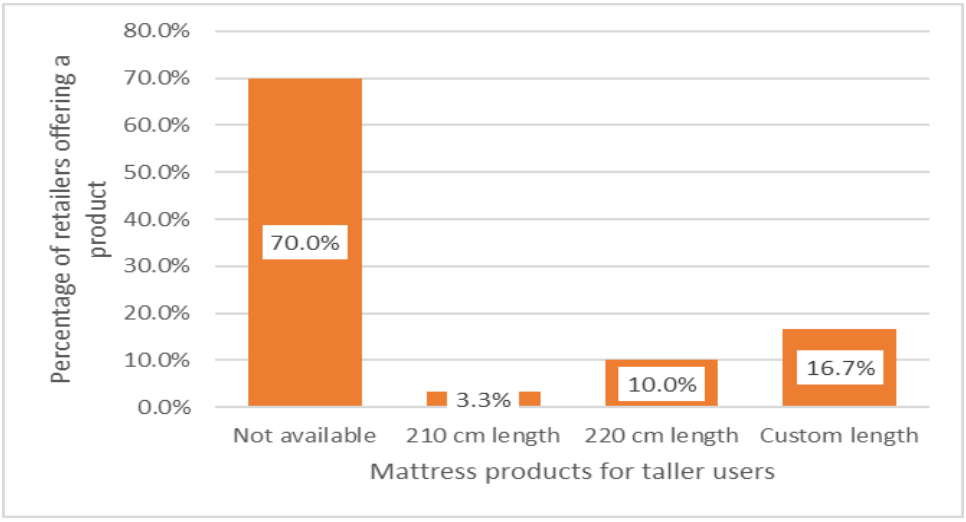

Figure 2. Availability of mattresses for taller users

When it comes to the filtering process, most retailers (60%) offer a firmness filter that allows the user to choose from predefined firmness levels. Sometimes, it's only three levels – soft, medium, and firm, however, some retailers include up to five levels of firmness in their filtering options. A similar situation was observed when it comes down to choosing a mattress based on its length, where 57% of retailers offered an option that would enable to activate a filter based on mattress size. Only 27% of retailers offered a filter setting that would display mattresses by the recommended weight of a user. Some retailers had these options in their mattress menu, too but it was a minority (10% for weight options and 17% for size options). Details are shown in Figure 3.

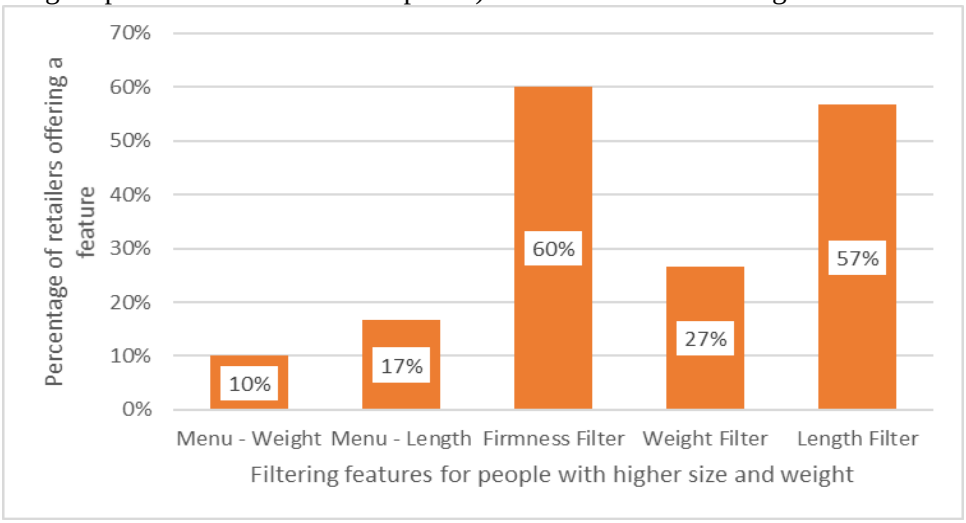

Figure 3. Options and settings of retailers' websites for filtering mattresses by parameters

In the following part, research questions defined above will be answered. The first research question (RQ1) was: Do Slovak retailers offer mattresses for heavier people above 110 kgs? The analysis has revealed that not although not every retailer offers mattresses for heavier people, the offer is quite rich. For example, 83% of the examined retailers offer an appropriate mattress for users weighing 130 kg. The choice however gets more limited with increasing user weight and gets quite limited with weight over 160 kg. There are, however, some options even for customers weighing 200 kg.

The second research question (RQ2) was: Do Slovak retailers offer mattresses for extra tall people? Unlike by heavier people, the research has revealed that the options here are quite limited. 70% of retailers only offer mattress with a standard height 200 cm. There are, however, options for taller people, with some retailers stocking mattresses 210 cm and 220 cm heigh and others offering custom tailored mattresses manufactured according to specific requirements.

Lastly, how easy/difficult is it for Slovak consumers to find such products online? (RQ 3). Based on the data presented in Figure 3 we can confirm that it can be quite challenging for people with non-standard weight and height to find an appropriate mattress. Most of the retailers do not offer filtering options based on the recommended weight of mattress user and therefore, the customer often needs to manually check every single product available and hope that at least on the product detailed page, such information will be displayed.

CONCLUSIONS

The following recommendations to retailers can be made in regards to their marketing, superficially, product portfolio and the way they present it online on their websites: i) Consider the basic anthropometric information of their customers – height and weight – and display this information by every product; ii) Consider adding more products into the mattress portfolio that would be suitable for taller people and overweight people; iii) Work on the user experience when looking for such products to shorten the selection process by enabling filters based on the height of the mattress and maximum recommended user weight.

This research has certain limitations that can also represent ideas for future research: i) The research has specifically focused on mattresses, however, other products such as beds, sofas or chairs also need to reflect changing anthropometric data of the population and could be subject to a similar analysis; ii) Only retailers from Slovakia were included in the research sample – if other countries are included, a comparative analysis could reveal interesting findings.

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Short Communication

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HOW ENSURE *IN VITRO* POLLEN GERMINATION OF CULTIVATED FLAX AND ITS WILD RELATIVES

SUMMARY

Until recently, flax was considered a crop whose pollen does not germinate well under artificial conditions. At the same time, rapid acquisition of information on pollen quality is important in many studies. Two linseed varieties and its three wild relatives with n = 15 were used as pollen sources. The basic artificial nutrient medium for pollen germination consisted of 200 mg/L boric acid and 200 mg/L calcium chloride. Experimental treatments additionally included polyethylene glycol of various molecular weights and sucrose. The media with polyethylene glycol 2000 in concentrations of 20-30 % ensured good pollen germination of cultivated flax, showing about 40% of pollen grains with normal pollen tubes. For wild species pollen, 20 % osmotic concentration was more preferable. The addition of sucrose to a medium with polyethylene glycol 2000 disadvantageously influenced pollen germination of cultivated flax and did not affect this indicator for wild species. When polyethylene glycol of higher molecular weight was used in a nutrient medium as an osmotic, the germinated pollen grains usually had burst pollen tubes or did not germinate at all.

Keywords: cultivated flax, wild species, pollen, *in vitro* germination, osmotic agent

INTRODUCTION

Flax (*Linum usitatissimum* L.) is one of the oldest cultivated plants, whose products have long been used for a variety of human needs. Two long-standing main directions of using the products of this plant have formed two different industrial crops – fiber flax and oil flax (linseed). Now each of these crops is grown on millions of hectares and a separate breeding is carried out for each of them. Some wild annual species with n = 15 of the *Linum* genus are actively involved in the breeding process when creating new varieties as the donors of

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many morphological, physiological and biochemical traits for cultivated flax (Jhala *et al.,* 2008; Lyakh and Soroka, 2008).

A very broad use of cultivated flax and its wild relatives provides for knowledge of the quality of pollen, produced by the plant. Pollen quality is important in many aspects. It influences seed set and ensures high seed yield. Again, when breeding programs for inter- and intra-species hybridization are laid out, it is necessary to evaluate pollen quality. Pollen could be also used to estimate some characteristic responses of mature plants to biotic and abiotic stresses at early stages of development and at the same time pollen germination could serve as a sensitive and simple bioassay for environmental quality. Thus, *in vitro* pollen germination technique was used by Hebbar *et al.* (2018), Karim Sorkheh *et al.* (2018) to screen genotypes tolerant to high temperature or to elucidate the genotype response at various temperature levels. In the studies of Lyakh *et al.* (1998), Patil *et al.* (2006), Lyakh and Totsky (2014) pollen was used to select valuable genotypes at the level of male gametophyte as well.

The ability of pollen to grow and germinate on an artificial medium allows estimating its quality fast and effectively. According to Golubinsky (1974) pollen of some plants needs only water to germinate. Pollen of most plant species, however, requires for its germination the presence in the nutrient medium of an osmotic agent, represented most often by sucrose (Baloch *et al.,* 2001; Liu *et al.,* 2013). Analyzing the composition of nutrient media for *in vitro* pollen germination in more than 800 plant species, D. Tushabe and S. Rosbakh (2021) note that sucrose is a component of such media for 89 % of species belonging to various genera and families. According to these and other authors, the other two most commonly encountered components of culture media are boric acid and calcium (Wang *et al.* 2003; Ahmad *et al.* 2012; Wani *et al.* 2020). In some plants, success in germinating pollen *in vitro* is ensured by changing the pH-value (Zaman, 2009; Acar *et al.* 2010). At the same time, it is known that not only sucrose can act as an osmotic agent. Instead of sucrose polyethylene glycol of the certain molecular weight can be added to a germination medium. Sometimes artificial nutrient media for pollen germination can include sucrose in addition to polyethylene glycol (Jayaprakash, 2018).

In *Linum usitatissimum*, separate attempts were undertaken to germinate pollen *in vitro* (Pandey and Kumar, 2013). However, the nutrient medium they used, in which sucrose acted as an osmotic, did not favor normal pollen germination. To date, for the pollen of both cultivated flax and its wild relatives, the nutrient medium, which ensures the emergence of properly-shaped pollen tubes during pollen germination, has not yet been developed. In this respect we supposed that polyethylene glycol could be used as an osmotic instead of sucrose taking into account that it promotes pollen germination in some species. The purpose of this study was to select for the basic nutrient medium, containing boric acid and calcium chloride, an osmotic agent that provides successful germination of pollen in *Linum usitatissimum* L. and some wild species which easily cross with the cultivated flax.

MATERIAL AND METHODS

The research were conducted at the Institute of Oilseed Crops of the National Academy of Agrarian Sciences of the Ukraine (IOC NAAS) during 2020 year. Two linseed varieties and three wild species of the *Linum* genus with *n* = 15 (*L. angustifolium* Huds., *L.bienne* Mill., and *L. hispanicum* Mill.) from the collection of IOC NAAS were used in the studies as pollen sources.

Boric acid and calcium chloride at the concentration of 200 mg/L, as the most frequently included chemicals for *in vitro* pollen germination, were mandatory components of all tested media (Jayaprakash, 2018). As an osmotic, polyethylene glycol (PEG) of various molecular weights or PEG in combination with sucrose were added to the basic medium containing boric acid and calcium chloride. The following nutrient media, differing in osmotic content, have been tested: PEG 2000, 20 % (a), PEG 2000, 30 % (b), PEG 2000, 30 % + sucrose, 5 % (c), PEG 2000, 30 % + sucrose 15 % (d), PEG 20000, 30 % (e), PEG 20000, 5 % (f).

Pollen was collected at early hours from 7 to 9 a.m., out of 20-40 flowers. Freshly collected pollen was germinated for 3-4 hours in a drop of an artificial medium placed on a slide at the temperature of 25±1°C in the dark. After that, the pollen was viewed under a light Leica microscope (Germany) with a 20^X objective. Pollen grains were counted as germinated if the pollen tube length was more than a pollen grain diameter. Pollen grains with not-burst (normal) and burst tubes were recorded separately. In each 5 replications of each treatment from 300 to 400 pollen grains were analyzed (Lyakh and Soroka, 2008).

The results of the experiments were analyzed statistically at the IOC NAAS and significance level was calculated applying a *t*-test, according to Wasserman (2005).

RESULTS AND DISCUSSION

A starting medium consisted only of boric acid and calcium chloride dissolved in distillate water. Since flax pollen did not germinate on such a medium, it was decided to supply it with an osmotic agent. In the preliminary experiments the liquid medium where only sucrose was used as an osmotic agent showed to be ineffective for pollen germination of the *Linum* species under study. The same adverse effect was observed when an agar-agar was added to the medium to solidify it. If the liquid medium contained PEG instead of sucrose in the concentration of 10 %, single pollen grains started to germinate.

On all the nutrient media tested in the experiment, pollen grains with both normal and burst pollen tubes were detectable among the germinated pollen. Although number of normal tubes in most cases considerably exceeded number of burst tubes, the ratio of both was significantly influenced by the composition of the nutrient medium.

Table 1 shows that a medium containing polyethylene glycol-2000 as an osmotic in the concentration of 20 % ensured a sufficiently good germination of the pollen for the species under study. The percentage of pollen grains with

normal (not burst) pollen tubes ranged from 39.1 % in *L. usitatissimum* to 22.7 % in *L. bienne*. The elevation in concentration of PEG 2000 from 20 % to 30 % reduced this indicator for wild species and did not affect pollen germination in cultivated species.

Table 1. Influence of polyethylene glycol 2000 concentration on pollen germination in *Linum usitatissimum* and its close wild species

Species	Polyethylene glycol 20 %		Polyethylene glycol 30 %	
	Normal pollen tubes, %	Burst pollen tubes, %	Normal pollen tubes, %	Burst pollen tubes, %
L.usitatissimum	39.1±2.77	25.1±2.47	38.5±2.74	21.0±2.29
L.angustifolium	44.8±2.80 [a]	7.6±1.49	8.3±1.59	2.3±0.87
L.bienne	22.7±2.43 [a]	5.5±1.33	9.9±1.82	2.2±0.88
L.hispanicum	24.3±2.66 [a]	6.9±1.57	12.1±1.95	3.8±1.14

[a] – differences between treatments are significant at the 0.1 % level of significance

It should be also noted that on both mentioned media the number of grains with burst pollen tubes was significantly smaller than the amount of normally germinated pollen grains in all species. However, if in wild species the proportion of pollen grains with burst pollen tubes was relatively small (15-20 % of all germinated grains), in cultivated flax *L. usitatissimum* it accounted for about 40 % of all pollen capable of germination.

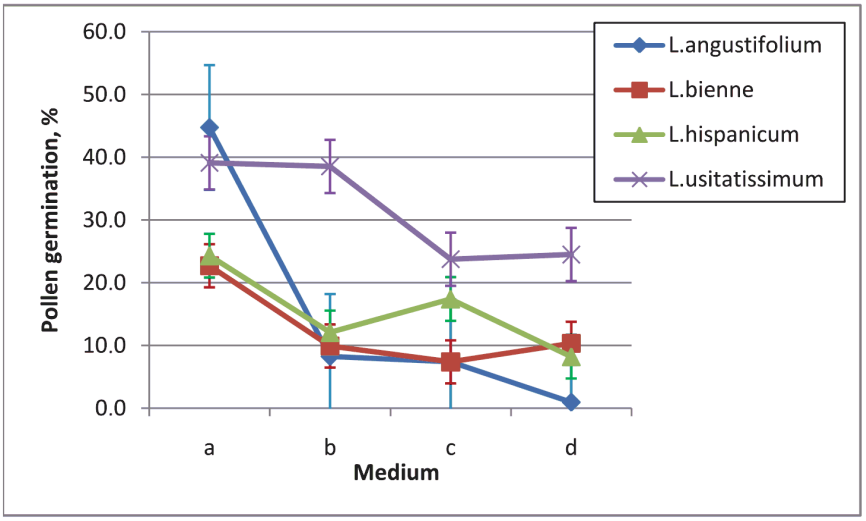

Fig. 1. Influence of PEG 2000 concentration and sucrose addition on the pollen germination of *L. usitatissimum* and its wild relatives with n = 15

Addition to the nutrient medium with PEG 2000 of such an osmotic agent as sucrose adversely affected pollen germination in *L. usitatissimum* (Fig. 1). The inclusion of sucrose at the concentration of 5 % and 15 % to a medium with PEG

30 % reduced the number of pollen grains with normal pollen tubes in this species by almost half. At the same time, in the closest wild relatives, the addition of sucrose to the medium with PEG 2000 did not adversely affect pollen germination.

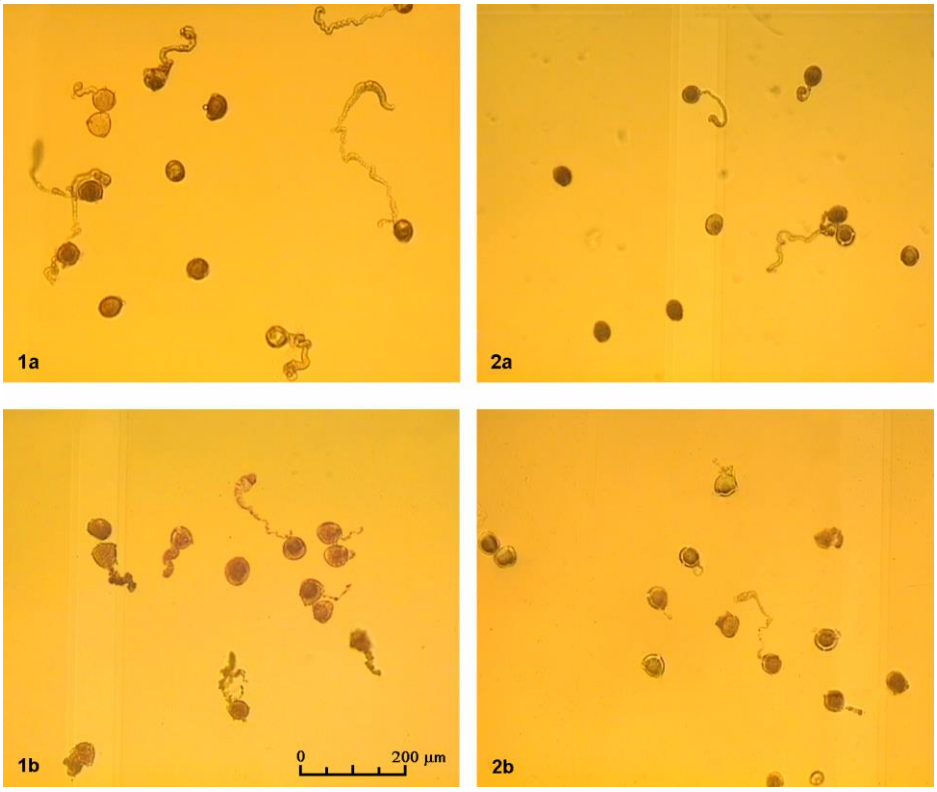

Fig. 2. Pollen germination of *L. usitatissimum* (1) and *L. hispanicum* (2) on the media with PEG of different molecular weight: (a) PEG 2000, 30 % (b) PEG 20000, 5 %

In addition to PEG 2000, PEG of higher molecular weight was tested as an osmotic in two concentrations – 30 % and 5 %. When the medium included PEG 20000 at the concentration of 30 %, pollen of all the studied species failed to germinate. Fig. 2 demonstrates the effect of PEG 20000 at the concentration of 5 % on pollen germination in *L. usitatissimum*, showing the large proportion of pollen grains with burst pollen tubes. When pollen was cultivated on the media, containing PEG 2000 at the concentration of 30 %, pollen grains formed predominantly normal pollen tubes.

Thus, a nutrient medium containing, in addition to boric acid and calcium chloride, an osmotic agent in the form of polyethylene glycol 2000 at the concentration of 20-30 %, can be proposed for evaluating the viability of pollen in cultivated flax and its closest wild relatives. Although in such a medium some of the germinated pollen has burst pollen tubes, the proportion of pollen grains

with normal pollen tubes is high enough to quickly and efficiently assess the quality of the male gametophyte or conduct some sort of selection at this level.

It is known that nutrient media in which sucrose is used as an osmotic agent are quite suitable for the germination of pollen from many plant species, including such well-known ones as, for example, tomato or maize (Jayaprakash, 2018). A similar medium involving only sucrose as an osmotic agent was also tested on flax (*Linum usitatissimum*). However, the abundance of burst and severely deformed pollen tubes developed under such conditions does not allow to assess the viability of pollen of this species in a proper way (Pandey and Kumar, 2013). Some experiments are known where sucrose was successfully combined with another osmotic agent (Jayaprakash, 2018). Thus, for the germination of sunflower pollen a nutrient medium was developed which included 15 % sucrose and 30 % polyethylene glycol 6000 (Keshava Murthy *et al.*, 1994). At the same time, our studies on flax demonstrated that addition of sucrose together with polyethylene glycol either significantly reduced pollen germination, as in cultivated flax, or did not positively affect the ability of pollen to germinate *in vitro*, as in the three wild flax homostyle species. In any of those cases, the addition of sucrose to the nutrient medium simultaneously with polyethylene glycol did not stimulate pollen germination and indicates the inefficiency of this osmotic agent for germination of pollen of any flax species.

It should be also noted that in our research polyethylene glycol at the concentration of 20 % and 30 % was equally effective for the germination of cultivated flax pollen, while the pollen of all three wild flax species reacted negatively to the presence of this osmotic agent in the nutrient medium at the concentration of 30 %, compared with 20 %. It can be assumed that those differences in the reaction of pollen are due to the different osmotic potential of pollen grains of wild species and cultivated flax, which is a consequence of their relatively asymmetrical morphological and physiological features (Jhala *et al.*, 2008).

CONCLUSION

Despite the fact that there are different methods for assessing the quality of pollen - from its germination *in vivo* on the stigmas of pistils to staining with various dyes – *in vitro* pollen germination on a nutrient medium is considered the fastest and most reliable. The revealed ability of pollen of cultivated flax and its closest wild relatives to germinate under artificial conditions makes it possible to control more effectively the success of crosses and determine pollen productivity, to develop methods for evaluating and selecting valuable genotypes at the microgametophytic level, as well as to carry out a number of other manipulations associated with the reproductive system of this important agricultural culture.

As follows from the results of the presented studies, the medium for *in vitro* germination of flax pollen, in addition to the standard boric acid and calcium chloride, commonly used in work with other plant species, requires the presence of an osmotic agent. Polyethylene glycol with a molecular weight of

2000 can act as such an osmotic agent. At the same time, while both concentrations of this osmotic (20 % and 30 %) are quite acceptable for cultivated flax, only 20 % concentration of polyethylene glycol is suitable for annual wild flax species. The use of sucrose together with polyethylene glycol not only does not improve the germination of flax pollen grains, but often inhibits this process.

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