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USE OF GAMMA IRRADIATION TO OBTAIN NEW FORMS OF WINTER WHEAT BASED ON LOCAL VARIETIES

SUMMARY

The research objective was to identify the peculiarities of the mutational activity induced by different doses of gamma irradiation in local winter wheat varieties based on the indicators of induced mutations spectrum in the second to fourth generation, to identify new high-potential forms in terms of grain productivity and quality, to identify variable components in forming such traits for a more controlled action by mutagenic agents. It has been established that a positive mutational process is essential for many useful traits, depending mainly on the original form genotype. The process has been proven to be a reliable permanent source of variability for the local genetic resources in terms of certain traits of economic value to obtain modern highly productive and highly adaptive potential forms. The regularity of the beneficial mutational process has been described, which allows to make the process of using this type of variability in order to obtain new material with the required potential more manageable, reliable and predictive.

Keywords: winter wheat, gamma irradiation, mutation spectrum, mutagenesis.

INTRODUCTION

The effect of physical mutagens on plants usually results in hereditary mutations, which can be beneficial for practical use in agriculture. Even a small single exposure significantly corrects the vitality and heredity of the plant (Daryanto *et al*, 2017).

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Gamma irradiation is a popular mutagen of the kind, used for both mutational improvement and studies on the effects of mutagens on living organisms (including model objects). The overwhelming number of successful obtainment of practically valuable varieties of agricultural crops is due to the use of gamma irradiation, which, taking into account the high intensity of relevant research, is still widely used both in traditional breeding improvement, and for the studies of systems of genetic control of certain features and obtaining of fundamentally new architecture of the stem and head in wheat. Thus, in the 21st century, 217 varieties were created by irradiation: China – 93, the USA – 18, Russia and Iraq – 13, India – 10, Bangladesh and Pakistan – 9, Vietnam – 5, Poland – 2, Japan and Korea – 7. 62.2% of varieties were created in Asia, 11.7% – in Europe (Pandit *et al*, 2021).

Some researchers believe that it is better to use high doses of gamma irradiation (within the range of 200–300 Gy). Existing FAO/IAEA statistics (Prabhu, 2019) partially supports the statement. Genetic improvement using different doses of gamma irradiation has been successful in creating productive mutants, resistant to disease and abiotic stress. Gamma irradiation quite often leads to drastic morphological changes in organisms. It is mutants with dramatic phenotype changes that should have beneficial changes in the content of valuable biochemical substances (Žofajová *et al*, 2017).

The effect of gamma irradiation is characterized by pronounced mutations, a high number of morphoses and genocopies. Although it is not the best choice in terms of increasing mutational activity, gamma irradiation of dry seeds is more effective in view of subsequent obtaining of mutational material, which has become an established practice worldwide. The increase in frequency and spectrum in the case of other subjects is insignificant due to further problems (Shan *et al*, 2018).

Studies on the experimental mutagenesis of cereal crops in South-East Asia, according to international scientific cooperation programs, have resulted in over 30 new varieties of crops obtained through the use of gamma irradiation. Physical mutagens are used more frequently and more efficiently (Tsenov *et al*, 2015; Liu *et al*, 2017).

The doses of gamma irradiation are considered optimal, when the seed similarity is 70-80%, and the survival of plants is 80-90%. That is, gamma irradiation of 100 - 150 Gy (Nazarenko *et al*, 2019b). It is believed that low-dose gamma irradiation changes the ratio and interaction of various valuable traits in crops quite significantly, and generally increases productivity, quality, and adaptive potential (Katyal *et al,* 2016; Datsu *et al,* 2020). As a result, mutants were obtained with practically valuable traits in terms of yielding, with an increased content of essential amino acids and microelements. When using small doses of gamma irradiation during certain periods of growth and development, when a new trait is formed and with the corresponding effect of environmental factors, a mutation occurs as a process of formation and leads to stable and practically valuable traits (Polatovich *et al,* 2021).

The main purpose was to identify the peculiarities of the level of mutability for local winter wheat varieties based on the indicators of mutations rate and spectrum at the second - fourth generations, the possibilities of local genetic sources for improvement through mutation breeding methods.

The main tasks were: to study the indicators of the rate of several value groups of mutations, their availability in the spectrum of mutations for local winter wheat varieties Komertsiina and Spivanka in the second - fourth generations; to identify the effect of individual doses of gamma-irradiation on the spectrum of induced changes, genotype and mutagenic interaction; to analyze the mutation spectrum, identify its key components and the possibility of obtaining new agricultural- and genetic-value forms; to identify the high-productive and high-qualitive (protein content and protein components) lines as future commercial varieties or components for hybridization.

MATERIAL AND METHODS

The research was carried out on the experimental fields of the Educational and Scientific Center of the Dnipro State Agrarian and Economic University in 2015-2021. The experimental areas have a homogeneous cover, consisting of ordinary low-humus, leached, medium-loamy black soil on a loamy soil. The content of nitrogen (according to Tiurin) during the years of research has not exceeded 3–5 mg, mobile phosphorus (according to Chyrykov) – 20-30 mg, exchange potassium (according to Chyrykov) – 20-35 mg per 100 g of dry soil.

The experimental field is located in the Dniprovskyi district of the Dnipropetrovsk region, Ukraine, which is the northern warm and insufficiently wet area. Its climatic resources are characterized by the following indices: hydrothermal coefficient is >0.9, precipitation rare during the growing season – 250-280 mm, the annual amount of precipitation – 450-490 mm, temperature sum for the period with the temperatures above 10°C – about 2,900°C.

As a material for the study, the following varieties were used: local varieties of Komertsiina and Spivanka (Dnipro State Agrarian and Economic University), being the material that fully meets the conditions of the region (Northern Steppe of Ukraine). Dry seeds were exposed to gamma irradiation of 100, 150, 200, 250, 300 Gy. Doses of gamma irradiation are standard for the irradiation spectrum used in the experimental mutagenesis of this culture to increase the variability of the starting material.

The variability rate was calculated using the formula

$Pv = \alpha \bullet \gamma$,

where Pv is the variability of the variant;

α is the ratio of the number of mutations to the total number of families in the variant;

γ is the number of types of changed traits in the variant.

The seeds of these varieties were treated at the gamma-ray unit of the Nuclear Research and Training Centre of the Department of FAO/IAEA Joint

Division of Nuclear Techniques in Food and Agriculture (Austria, Freiburg), with gamma rays of the Co60 radioactive source, the capacity of the unit is 0.048 Gy/s.

In the second and third generation, we studied the mutations visually and by yield at hand sowing by families (1-3-row plots, row spacing of 0.15 m, row length of 1.5 m), carried out inheritance studies, productivity and structural analysis of selected mutant strains (plot area of 5 – 10 m^2, 1-3-time repeatability). The mutation rate was calculated as a ratio of mutant cases to the total number of families in the second generation in percent, the variability was measured as a ratio of changed traits to the proportion of mutant families in the second generation – each mutant case as an individual phenomenon. Mutant strains (25 plants each) were analyzed to measure yield parameters. The area of the plots was 5–10 m^2 depending on the year of the experiment, the repetition was 1-2 times, the standard – every 20 numbers. Two performance and quality reference groups were seeded – the original form and the national standard of Podolyanka variety. The protein content in wheat grain was measured on the Spektra RT device, the content of glutenins and gliadins by liquid chromatography on the RP-HPLS device

Statistical processing of the obtained results was carried out using the discriminant analysis, the quality of the mean difference was assessed by factor analysis. The standard package of the Statistic 10.0 application was used.

RESULTS AND DISCUSSION

Indicators of variability of families and lines in M_{2-4} induced by different doses of gamma rays, are given in Table 1 (total rate of mutations, number of changed traits, variability). In the Komertsiina variety, the mutation rate ranged from 8.40% (gamma rays, 100 Gy) to 30.00% (gamma rays, 250 Gy) with a 1.20% control (spontaneous mutation rate). In the Spivanka variety, 6.40% (gamma rays, 100 Gy) to 31.67% (gamma ray, 250 Gy) at 0.80% of spontaneous mutations in the untreated reference group. As can be seen from the growing mutation rate in both varieties, it occurs gradually, but in general in the Spivanka variety, the rate is significantly lower than in the Komertsiina variety, with a sharp increase in this parameter at a dose of 250 Gy (almost 60% of the previous number), while in the Komertsiina variety the mutation rate remains mostly unchanged at doses of 200–250 Gy and is not characterized by peak increases.

The number of types of changed traits within the entire dose range is higher, in contrast, in the Spivanka variety, which characterizes a significant increase in the spectrum of mutations for this genotype, while in the Komertsiina variety the number of mutants in terms of individual traits increases significantly. However, the overall spectrum is substantially scarce. Depending on the specific content of the range of changes, this may mean that at a high level of variability in terms of economic value, this variety may be more viable directly for obtaining economically valuable forms. Whereas the Spivanka variety is more effective in producing collections of genetically valuable traits, followed by use for improvement through combinatorial variability. In any case, in the Komertsiina variety, the number of types of changed traits decreases at increasing the gamma irradiation doses, while in the Spivanka variety, the spectrum, on the contrary, expands to a dose of 150 Gy, followed by a decline in doses of 200–250 Gy.

Table 1. Mutation rate and variability of winter wheat due to the action of gamma irradiation

Variety	Gamma ray dose				
	Control	100 Gy	150 Gy	200 Gy	250 Gy
Rate					
Komertsiina	1.20±0.11	8.40±0.62^{b}	13.56±1.05^{c}	29.17±1.41^{d}	30.00±1.69^{d}
Spivanka	0.80±0.11^{a}	6.40±0.53^{b}	10.75±0.92^{c}	19.00±1.14^{d}	31.67±1.54^{e}
Modified Traits					
Komertsiina	4^{a}	21^{b}	18^{b}	15^{c}	11^{d}
Spivanka	4^{a}	24^{b}	28^{b}	24^{c}	15^{d}
Variability					
Komertsiina	0.05^{a}	1.76^{b}	2.45^{c}	4.38^{d}	3.30^{e}
Spivanka	0.03^{a}	1.54^{b}	3.02^{c}	4.56^{d}	4.74^{d}

Note: The difference is statistically significant at P0.05 taking into account the Bonferroni correction

The variability rate, as a complex indicator of genotype mutability due to a wider spectrum, is higher in the Spivanka variety, although not always significantly, and increases constantly with increasing dose, although not always significantly (250 Gy dose). The variability in the reference group is negligible, further indicating the spontaneous nature of the mutations obtained.

According to the results of factorial analysis, it was found that only the "dose" parameter was affected by the overall rate of mutations ($F=29.51$; $F_{critical}=6.39$; $P=0.01$). That is, despite significant differences, the nature of the variety ($F=1.89$; $F_{critical}=7.71$; $P=0.24$) did not in any way affect this indicator and the differences as a result of different doses, which is not enough to assess the genotype and mutagenic interaction factor as significant as a whole.

The index of variability showed that changes in frequency together with the differences in the breadth of the mutation spectrum were already significantly depended not only on the dose applied ($F=30.06$; $F_{critical}=6.39$; $P=0.01$), but also on the genotype ($F=9.76$; $F_{critical}=7.70$; $P=0.03$) – that is, on the grade of the starting material. The effect of the dose difference on mutational variability in both cases remains sound and significant.

In terms of the spectrum of action of gamma rays, 33 types of changed traits have been identified (which basically is not that much for the action of gamma irradiation as a mutagen of systemic, nonspecific action) and classified into the following groups: I. Stem and leaf structure mutations – all changes in stem and leaf morphometry and morphology. 1 Thick stem. 2. Thin stem. 3.

High-stem. 4. Short-stem. 5. Semi-dwarf. 6. Dwarf. 7. Intense epicuticular wax accumulation. 8. Mild epicuticular wax accumulation. II. Mutations in grain colour and structure. 9. Large-size grain. III. Mutations in spike colour and structure. 10. Awned spike. 11. Awnless spike. 12. Long spike. 13. Loose spike. 14. Cylindrical spike. 15. Spindle-shaped spike. 16. Compact spike. 17. Large-size spike. 18. Small-size spike. 19. Half-awned spike. 20. Rigid spike. 21. Club-shaped spike. 22. Arrow-shaped spike. 23. Anthocyan awns. IV. Altered physiological signs of growth and development. 24. Sterility. 25. Early ripeness. 26. Late ripeness. V. Mutations in grain productivity and quality. 32. Productive. 33. Forms with high tillering capacity. VI Systemic mutations are mutations beyond the systematic features specifically attributed to common winter wheat and more are more peculiar to related forms. 27. Square-head spike. 28. Speltoid spike. 29. Subcompactoid. 30. Compactoid. 31. Spherococcoid.

According to the results of the discriminant analysis (Table 2, only significant traits are given), the following key features can be distinguished, which are standard for both varieties: high-stem, short-stem, semi-dwarfs, intense wax accumulation, mild wax accumulation, awned spike, awnless spike, long spike, large-size spike, sterility, late ripeness, early ripeness, tillering capacity, productive, speltoid spike (a single systemic feature), a total of 15 traits from 33.

Table 2. Classification matrix by canonical roots

Criteria	Availability in the model			Percentage of classification	
	Wilks' lambda λ	$F_{critical}$ (3.02)	p-level	Komertsiina	Spivanka
high-stem	0.32	1.96	0.01	100.0	100.0
short-stem	0.25	2.13	0.01	79.0	88.0
semi-dwarf	0.20	2.86	0.02	21.0	70.0
intense epicuticular wax accumulation	0.24	2.22	0.01	100.0	100.0
mild epicuticular wax accumulation	0.19	2.91	0.03	85.0	79.0
awned spike	0.18	2.95	0.04	73	--
awnless spike	0.18	2.97	0.04	--	60.1
long spike	0.18	2.90	0.04	70.0	61.0
large-size spike	0.18	2.94	0.04	61.0	58.0
sterility	0.17	2.99	0.05	33.0	67.0
late ripeness	0.21	2.72	0.02	100.0	100.0
early ripeness	0.18	2.98	0.04	79.0	76.0
tillering capacity	0.18	2.94	0.04	67.0	64.0
productive	0.18	2.94	0.04	61.0	58.0
speltoid spike	0.17	3.01	0.05	58.0	67.0
Average	--	--	--	68.2	72.9

Classification capabilities were analyzed in terms of significant features for individual genotypes (since awnless forms are only available in the Spivanka variety, awns are a mutation for the Komertsiina variety, as well as to detect genotype specificity in the gamma irradiation).

According to the above data, such features as a semi-dwarf and sterility fall out of the classification model for the Komertsiina variety. For the Spivanka variety, the classification significance of all traits is maintained, however with some minor changes. In general, the chances of modeling the mutation process for the Spivanka variety are slightly higher, but not significantly.

The greatest classification ability is found in such signs as plant height mutations (primarily tall-stalked), changes in the period of ripeness and wax accumulation. Of these, short-stalked and early-ripeness mutations are of practical importance.

11 viable mutant lines (third to fifth generation) were tested for yield, elements of its structure and grain quality. 6 lines were obtained from Spivanka variety and 5 from Komertsiina variety.

Data on the three-year yield test are given in Table 3. As we can see, only the lines (except line 203) that exceeded the Podolyanka variety standard during the test were selected.

Table 3. Yielding capacity of agrocenoses of mutant strains of common winter wheat during the experiment

Genotype	2018	2019	2020	Average	+/- to standard
	t/ha				
Podolyanka, st.	5.23±0.09	5.42±0.07	7.89±0.12	6.18±0.10	
26	7.39±0.15*	6.05 ±0.09*	8.11±0.16	7.18±0.14*	1.00
45	7.51±0.16*	6.22±0.13*	8.09±0.15	7.27±0.13*	1.09
123	7.22±0.11*	6.10±0.08*	8.13±0.15*	7.15±0.14*	0.97
152	7.11±0.15*	6.09±0.08*	8.15±0.14*	7.12±0.14*	0.94
178	7.01±0.13*	6.00±0.07*	8.69±0.17*	7.23±0.15*	1.05
179	7.61±0.17*	6.31±0.09*	8.19±0.16*	7.37±0.15*	1.19
181	6.40±0.09*	6.22±0.08*	8.22±0.18*	6.95±0.13*	0.77
203	6.09±0.08*	5.92±0.07*	7.17±0.14	6.39±0.11	0.21
213	6.44±0.09*	6.12±0.08*	7.43±0.15	6.66±0.13*	0.48
214	6.65±0.10*	6.14±0.08*	7.32±0.14	6.70±0.13*	0.52
262	6.43±0.09*	6.36±0.09*	8.29 ±0.16*	7.03±0.14*	0.85
$HIP_{0.05}$	0.22				

** - statistically significant difference from the Podolyanka standard at $P_{0.05}$*

However, under optimal conditions (the most successful for the fulfillment of productive potential) in 2020, some lines formed yields at the standard level, which indicates their reference to a semi-intensive type with wider variability limits and that they are hardly better in terms of productivity than the original lines (for lines originating from the Komertsiina variety – lines 203, 213, 214) or are even worse (for lines originating from the Spivanka variety – lines 26 and 45). Thus, the lines 123, 152, 178, 179, 181, 262 can be considered more successful and recommended as the top priority. 4 lines are derived from the Spivanka variety and 2 from the Komertsiina variety. Thus, from the standpoint of assessing variability in terms of productivity trait, the use of Spivanka variety as the original form was more successful. This once again proves that it is the greater degree of variability and perfection in that particular trait that are more important factors in improving a certain line.

The results of factor analysis according to the dispersion analysis scheme confirm this fact. Thus, the genotype factor ($F=4.66$; $F_{critical}=4.11$; $P=0.02$) was significant, however, the year factor ($F=70.35$; $F_{critical}=3.44$; $P=0.01$), which caused a significantly greater proportion of dispersion in productivity variability, turned out to be more important.

Above all, this indicates the achievement of the necessary contrast and variability of the conditions of the years, which made it possible to fully analyze this trait. It also proves that weather conditions still remain the main limiting factor and it is impossible to significantly compensate for their effect solely on account of genetically determined adaptability.

Factor analysis proved that it was the interaction of the conditions of the year and the variety genotype that led to the differentiation of the original material, and showed that the factor of the original material genotype plays essential part, but it is obviously difficult to identify this role in more detail.

It was decided to analyze the elements of the yield structure within the framework of the study and by detailing the mechanism of agrocenoses productivity at the level of individual elements, to carry out a more detailed factor analysis to identify the priorities of factors and establish the most determinative aspects. The synergy of individual economic features was also interesting in the context of formation of the total yield and possible changes in the model of influence of individual traits (while the Spivanka variety is more oriented to productive tillering capacity, the Komertsiina variety – to grain content and filling of the main spike).

To determine the factors increasing yields, its structural analysis was performed (Table 4) by the following indicators: height of plants, productive tillering capacity, number of main spike grains and grain weight, plant grain weight, thousand kernel weight (TKW). As a result, it was found that in all productive lines, the increase in yield was influenced primarily by the increase in TKW and the grain weight of the plant, which was less than the grain weight of the main spike. As we can see, there were changes in the formation partly for each of the two original forms due to individual traits of the total yield at the level of the grain weight of the plant and the grain weight of the spike.

Table 4. The main indices of the yield capacity structure of mutant strains of common winter wheat

Line, variety	Height of plants	Productive tillering capacity	Number of grains per main spike	Grain weight per main spike	Grain weight per plant	Thousand kernel weight
	cm		pcs.	g		
Podolyanka	103±2.0	4.0±0.1	36.5±1.6	1.9±0.2	4.3±0.2	50.2±1.0
26	83.8±2.1*	3.9±0.1	42.2±2.4*	2.4±0.2*	4.9±0.3	51.1±0.9
45	82.4±2.1*	3.6±0.1	38.1±2.4	2.5±0.2*	4.4±0.3	52.9±0.9*
123	74.3±2.1*	3.6±0.1	42.6±2.4*	2.0±0.2	4.9±0.3*	52.3±0.9*
152	76.5±2.0*	4.0±0.1	38.7±2.4	2.2±0.2	5.1±0.3*	53.1±0.9*
178	74.7±2.0*	3.8±0.1	37.5±2.4	1.9±0.2	5.2±0.3*	50.9±0.9
179	59.4±2.0*	3.7±0.1	37.2±2.4	1.7±0.2	4.6±0.3	52.4±0.9*
181	102.2±1.5	3.4±0.1	38.9±1.8*	2.3±0.2*	4.7±0.2*	53.5±0.8*
203	101.6±1.6	3.5±0.1	37.0±1.8	1.9±0.2	4.8±0.2*	50.5±0.8
213	99.8±1.5	3.4±0.1	37.9±1.8	2.1±0.2	4.8±0.2*	52.5±0.8*
214	78.8±1.6*	3.3±0.1	39.0±1.8*	2.4±0.2*	4.4±0.2	53.1±0.8*
262	59.6±1.5*	3.6±0.1	36.1±1.8	2.4±0.2*	4.4±0.7	53.9±0.8*

** - statistically significant difference from the Podolyanka standard at* $P_{0.05}$

Thus, it can be stated that the increase in yield can be both due to changes in the mechanism of yield capacity formation as compared to the previous form, and to the enhancement of the trait being the key one for the original form. It is impossible to identify more precisely the priority here, so obviously, large-scale studies are necessary in terms of the amount of material received at this point, so a factor analysis was performed (Table 5) in order to find out the most important prerequisite for increasing yield capacity: either variability due to the effect of gamma irradiation of individual elements of the yield structure or the perfection of the original genotypes (this is also important in view of the conclusion about the viability of certain forms as an original material).

As a result of factor analysis, it was established that the genotype conditioned a priority in the formation of such traits as productive tillering capacity and number of grains per main spike, that is, it was not possible to provide a significant level of such traits variability through the use of the mutagen. Variability due to the stem height (which affected the same coefficient of economic use – the ratio of the economic and vegetable parts) was conditioned by gamma irradiation only.

This suggests that it is the height, grain weight per spike and plant, and TKW that can be improved with gamma irradiation relatively easily, while other indicators are much harder to enhance. It also indicates the prospect of using more advanced local forms.

Table 5. Factor analysis findings (varimax raw)

Parameter	Genotype	Gamma irradiation
Height of plants	0.218111	**0.544179**
Productive tillering capacity	**0.532458**	0.176666
Number of grains per main spike	**0.516141**	0.075987
Grain weight per main spike	**0.471919**	**0.117111**
Grain weight per plant	**0.521670**	**0.683080**
Thousand kernel weight	**0.492827**	**0.519850**
Total variance	2.753126	2.116873
Proportion of total variance	2.535015	1.86422

Note: The numbers in bold are statistically significant at $P_{0.05}$

As mentioned above, increasing productivity alone is not a necessary prerequisite for the creation of a satisfactory crops agrocenosis from the standpoint of today's needs (Jaradat, 2018; Lykhovyd, 2021). The second necessary component is the technological qualities of the grain, which are due to such hereditary features as the protein content in the grain, the content of gluten, availability of glutenins and gliadins and their complex in relative content (Khalili *et al,* 2018). The viable forms are those with a protein content of 14% and higher, with a higher content of high-molecular weight glutenins, and with no increase in the low-molecular weight component (Nutall *et al,* 2017).

The results of the study by quality parameters for all high-productivity strains are shown in Table 6. According to the coefficient of the trait variation, the protein content and the total gluten content were referred to low-variable – that is, it is quite difficult to achieve significant changes for them, other traits were medium-variable and more favourable to the induction of new values for these parameters.

Table 6. Technological properties of grain

Variety	Protein, %	Gluten, %	Glutenins			Gliadins
			HMW	LMW	Total	
Podolyanka	13.99	25.59	0.16003	0.46485	0.62488	0.4598
26	13.55	23.99	0.20443*	0.48435*	0.68878	0.3565
45	12.88	22,23	0.21245*	0.49453*	0.70698	0.3453
123	14.21*	27.15*	0.24353*	0.45467	0.6982	0.4325
152	13.95	25.66	0.21356*	0.45465	0.66821	0.4231
178	13.57	24.54	0.20435	0.47100	0.67535	0.4324
179	14.27*	27.17*	0.21231*	0.44454	0.65685	0.4657
181	14.14*	26.98*	0.21523*	0.50333*	0.71856	0.4456
203	13.13	23.11	0.19022*	0.51760*	0.70782	0.4200
213	13.92	24.99	0.18453	0.54300*	0.72753	0.5048*
214	13.55	24.18	0.18453	0.53999*	0.72452	0.4448
262	14.32*	27.62*	0.20444*	0.53545*	0.73989	0.4948*
Average	13.79	25.24	0.20247*	0.49233*	0.69480	0.4354
C_v, %	3.3	7.2	10.3	7.2	4.8	10.9

** statistically significant difference from the Podolyanka standard at $P_{0.05}$*

In terms of protein content, the standard was significantly dominated by such lines as 123, 179, 181, and 262. They were more advanced in terms of gluten content, which correlates with the protein content by a factor of 0.91. Lines 152 and 213 were as good as the standard forms. Other lines turned to be far worse. Unlike previous studies (Nazarenko and Izhboldin, 2017; Nazarenko *et al,* 2019a), the significance of a beneficial mutation process for such traits as semi-dwarfism (for individual genotypes) and stunting (Nazarenko *et al*, 2019b) has been shown, that is, it has been proved that the mutation is a reliable and permanent source for the local genetic resources (Hongjie *et al*, 2019) related to such traits, and that obtaining high-intensity stunting strains with a long grained spike (Keser *et al*, 2022) of early ripeness (Klčová *et al*, 2019; Kirova *et al,* 2021) and intense wax accumulation (Cann *et al*, 2022) (to eliminate the negative effects of drought) based on local material is quite possible and regular (Kirova *et al*, 2021).

The regularity of the beneficial mutational process has been described (Nazarenko and Bezus, 2018; Li *et al,* 2019), which allows to make the process of using this type of variability in order to obtain new material with the required potential more manageable, reliable and predictive (Nazarenko *et al*, 2021).

Based on the obtained material, it can be concluded that it is advisable to use mainly moderate doses of 100 – 150 Gy for the local material (Bondarenko and Nazarenko, 2020), and in some cases – a dose of 200 Gy (Nazarenko *et al*, 2019b).

For Steppe varieties, the combination of climatic factors and critical stages of development has become more favourable for the intensive (Western European) ecotype and seemingly allows less focus on local varietal resources. The studies show the key parameters of the existing mechanism of crop formation and the possibility of combining high yield capacity with quality grain based on local material due to the effect of gamma rays.

It should be recognized that it is possible and desirable to improve the local varieties for regional agriculture. Moreover, it should be noted that the obtained local varieties are as good as Western European varieties in terms of yield and quality. This cannot be considered as a positive trend, and the local breeding of new varieties of crops requires a significant increase in efforts. The key parameters for the formation of the yield capacity of local varieties are sufficiently flexible and variable for this purpose, and the quality has been sufficiently formed, although it is less mutable than the productivity traits.

CONCLUSIONS

Local genetic material is a quite favourable base for the creation of high-yielding and high-quality material. While combined with the relevant factor that induces genetic variability at a sufficiently high level, viable strains can be obtained with quite satisfactory results. It should be taken into account that the key productivity traits are easier to change than the technological properties of grain, so it is still advisable to pay particular attention to this aspect when assessing the original material. Classical factors for genetic improvement due to the mutational process remain effective in creating high-yielding and high-quality material. Further research will focus on key parameters such as drought resistance, photosynthetic activity, peculiarities of the use and accumulation of nutrients for the studied varieties to confirm the parameters that provide the identified advantage in terms of grain yielding capacity and quality indicators. It is also planned to study new factors that induce mutational changes based on local and international germplasm.

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STUDY ON THE CONTENT OF PHENOLIC COMPOUNDS, ANTHOCYANINS AND ANTIOXIDANT ACTIVITY OF RED GRAPES AND WINES FROM CENTRAL NORTHERN BULGARIA

SUMMARY

The content of phenolics and the antioxidant potential of grape must and wines from three varieties - Cabernet Sauvignon (introduced), Gamza (local) and Rubin (hybrid) grown in the terroir of the town of Pleven, Central Northern Bulgaria were investigated. The Rubin hybrid accumulated the most significant amount of phenols (TPC, FPC, NPC, and anthocyanins) identified in its grape must. This was reflected in the proved high antioxidant activity in the must of this variety. The highest content of TPC in the obtained wines from the studied varieties was found in Rubin. The results for the FPC of the wines tend to overlaped with the established anthocyanin content, namely in the order (Cabernet Sauvignon)>(Rubin)>(Gamza). It is well known that the anthocyanins are the part of FPC group. In this aspect our results were direct evidence that anthocyanins had a contribution to the formation of the total concentration of flavonoid phenolic compounds (FPC). The data on the established antioxidant activity in the red wines could be closely related to the NPC results of the wines. Gamza and Cabernet Sauvignon showed a close concentration presence of this group of phenols, which closely correlated with the values of the antioxidant activity found in their wines. The study proved high biological potential and good phenolic accumulation in grapes and wines of the studied varieties. The study enriches the field of viticultural and wine science with specific results on the biological potential of grapevine products from the *Vitis vinifera* L, under the conditions of a specific wine-growing region of Bulgaria.

Keywords: phenolic compounds, antioxidants, biological potential, grapes, wine.

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INTRODUCTION

The defining of the phenolic composition of grapes and wines from different regions of the world is a field of diverse research in food science (Yildirim *et al*., 2005). The content of phenolic compounds can serve as an indicator of the terroir influence (the complex influence of the soil-climatic zone where the varieties are grown) (Pajović-Šćepanović *et al*., 2018; Pajović-Šćepanović *et al*., 2019). The correlation between phenolic accumulation in grapes and wines and their antioxidant activity has been proven and confirmed in different studies (Ginjom *et al.,* 2010; Di Lorenzo *et al*., 2019; Mitrevska *et al*., 2020; Rodriguez-Vaquero *et al.,* 2020).

Ghatak *et al*. (2011) investigated the phenolic composition and antioxidant activity of white, rosé and red wines from India. For the reds, the team found a variation in the total content of phenolic compounds from 3.10±0.02 mg/ml to 6.50±0.10 mg/ml and a high antioxidant activity of 84.60±1.10%. Mucaca *et al.* (2017) investigated the phenolic composition and antioxidant activity of tropical Brazilian Syrah and Cabernet Sauvignon red wines (harvests 2011, 2013 and 2014) from the São Francisco Valley. The team found a variation in total phenol compounds from 1528.90 mg/dm^3 to 4003.96 mg/dm^3. The anthocyanins ranged from 98.94 mg/dm^3 to 501.20 mg/dm^3, and the antioxidant activity was found to range from 58.02% to 95.70%. Osorio-Macias *et al.* (2017) studied total phenolic compounds in South American red wines and found a variation in their content from 1600.00 to 3500.00 mg/dm^3. Čeryova *et al.* (2021) investigated the phenolic content of Slovak wines from muscat varieties. The team found total phenolic content in the wines ranging from 262.10 mg/dm^3 to 568.00 mg/dm^3, total flavonoid content ranging from 24.80 mg/dm^3 to 169.10 mg/dm^3, and established antioxidant activity between wines ranging from 25.20% to 67.70%. Ismael (2018) determined the total phenolic compounds, flavonoid phenolic compounds and antioxidant activity of grapes from 7 red grapevine varieties grown in the Kurdistan Region, Iraq. They found a variation in the content of total phenolic compounds from 122.77 to 249.19 mg GAE/100g, the variation in the content of flavonoid phenolic compounds was from 584.23 to 288.55 mg/100 g, and the established antioxidant activity ranged from 41.79% to 92.30%. Büyüktuncel *et al.* (2014) determined by spectrophotometric method the total phenolic content of wines from the trade network and found a variation in the content of total phenols from 2599.90 to 4867.57 mg/dm^3.

The diverse studies on the phenolic composition and antioxidant activity (biological potential) of grapes and wines from different regions of the world are the motivation for the aim of the present study – to characterize the phenolic composition and the antioxidant potential of grapes and wines obtained from red grapevine varieties grown in the area of the town of Pleven, Central Northern Bulgaria.

MATERIAL AND METHODS

Grapevine varieties

The object of the research were three red grapevine varieties (2021 harvest) - Cabernet Sauvignon (introduced), Gamza (local) and Rubin (hybrid), grown in the experimental plantations of the Institute of Viticulture and Enology (IVE), Pleven.

Cabernet Sauvignon – originating from the Bordeaux region of Southwestern France (Sweet, 2008). This variety has a control role in the research. For the Pleven region, it ripens in the second half of September. Its wines have intense ruby-red color, a variety-specific aroma and a dense taste. They have the aging potential (Radulov *et al.,* 1992; Roychev, 2012).

Gamza – it has a long history and popularity in Bulgaria and Romania. Its grapes ripen in the second half of September. The produced wines from it are characterized by bright red ruby color, delicate fruity aroma and don't have the aging potential (Radulov *et al*., 1992; Roychev, 2012).

Rubin – it was obtained through intraspecific hybridization by crossing Nebiolo x Shiraz (Petkov, 1977). For the Pleven region it ripens in the first half of September. The wines from it have intense dark red color, high extractability and harmonious taste (Radulov *et al*., 1992; Roychev, 2012).

Grape must chemical composition

The research of the chemical indicators was carried out according to the methods generally accepted in winemaking practice and includes: Determination of sugar content (g/dm^3) - hydrometrically, using a Dujardin hydrometer; Determination of the content of titratable acids (TA, g/dm^3) - by titration with 0.1n NaOH; Determination of pH - potentiometrically, using a pH meter.

Vinification

The studied varieties were harvested when they reached technological maturity. The grapes, in the amount of 30 kg, of each variety were processed in the Experimental Wine Cellar of IVE - Pleven, in the conditions of microvinification, according to the classic scheme for the production of red wines: Crushing - Destemming – Sulphitation (50 mg/kg SO_2) - Alcoholic fermentation (dry wine yeast Saccharomyces cerevisiae: 20 g/hl; temperature: 28°C) - Separation from solids - Additional sulphitation – Storage.

Wines chemical composition

The analyzes were carried out according to the methods generally accepted in the wine practice (Ivanov *et al*., 1979): The content of sugars (g/dm^3) - Schoorl's method; Alcohol content (vol. %) – distillation method using a Gibertini apparatus with a densimeter, by determining the density of a non-alcoholic sample; Titratable acids of the wine (TA, g/dm^3) – by titration with 0.1n NaOH; pH – potentiometrically with a pH meter; Total extract – by densimeter (Gibertini, Milan, Italy).

Grape must and wines phenolic content

Total phenolic compounds (TPC), g/dm^3 – determined according the method of Singleton et Rossi with Folin - Chiocalteu reagent (Ivanov *et al.*, 1979); Flavonoid phenolic compounds (FPC), mg/dm^3 - catechin equivalent - Sommers method (Chobanova, 2007); Non-flavonoid phenolic compounds (NPC), mg/dm^3 - coffee equivalent - Sommers method (Chobanova, 2007); Anthocyanins, mg/dm3 - method of Singleton et Rossi by pH changing (Ivanov *et al.*, 1979).

Grape must and wines antioxidant activity

The antioxidant activity was determined according to the method of Wang *et al.* (1996), as antiradical activity against the stable product DPPH (2,2 - diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany). The studied grape musts and wines were diluted immediately before analysis with distilled water to a total extract (TE) content of 600.00 and 400.00 mg/dm^3, respectively. 0.5 cm^3 of the grape must or wine and 2.5 cm^3 of a freshly prepared solution (100μm) of DPPH• in ethanol were mixed in the test tubes. Thus, the TE:DPPH ratio in the reaction medium assumed values of 3:1 and 2:1, respectively. Similarly, a control sample was developed with distilled water instead of diluted grape must or wine. The values of the molecular absorption of light (spectrophotometrically at a wavelength of 515 nm) of the control and experimental samples, denoted by Ak and Ae, respectively, were measured. The measurements were performed at a reaction times of 5^{th} and 15^{th} min, counted from the moment of reagents mixing. The antiradical activity was calculated by the formula:

$$AAR = 10^2.(Ak - Ae).Ak^{-1}, \% \quad (1)$$

Statistical data processing

Statistical processing of the data was performed, including determination of standard deviation (±SD), with triplicate replication for each analysis. The statistical data processing was carried out using the Excel 2007 program (Microsoft Corporation, USA).

RESULTS AND DISCUSSION

The harvest for each variety was carried out at technological maturity. Three main technological indicators were defined: content of sugars, titratable acids (TA) and pH. The obtained results are presented in Table 1.

Table 1. Main technological indicators of grape must from the studied varieties (harvest 2021)

Technological indicators	**Grapevine varieties**		
	Cabernet Sauvignon	***Gamza***	***Rubin***
Sugars, g/dm^3	235.60±2.500	242.00±1.700	265.00±0.000
TA, g/dm^3	9.54±0.190	4.90±0.100	6.20±0.110
pH	2.97±0.000	3.44±0.005	3.48±0.005

Rubin's sugar content was the highest (265.00±0.00 g/dm^3), followed by Gamza (242.00±1.70 g/dm^3). The lowest sugar accumulation was found in Cabernet Sauvignon (235.60±2.50 g/dm^3). According to Radulov *et al*. (1992) and Roychev (2012), the studied varieties in the conditions of Bulgaria should accumulate sugars as follows:

- Cabernet Sauvignon – 210.00 – 240.00 g/dm^3
- Gamza – 190.00 – 210.00 g/dm^3
- Rubin – 220.00 – 240.00 g/dm^3

It was clear from the data that only the Cabernet Sauvignon must felt within the range of variation proposed by the above authors. Gamza's must showed 32.00 g/dm^3 more sugars, and Rubin's 25.00 g/dm^3 more than the suggested range. The reasons for this were related to the influence of weather conditions during the year, as well as the characteristics of the growing area. The particular year was characterized by a very hot and dry summer, with a long period without precipitation, which led to an increased sugars synthesis in the grapes. Regarding the established titratable acidity (TA), Cabernet Sauvignon must was characterized by the highest content of it (9.54±0.19 g/dm^3). Rubin's must contained more titratable acids (6.20±0.119 g/dm^3) compared to Gamza (4.90±0.19 g/dm^3). According to Radulov *et al.* (1992) and Roychev (2012) the content of titratable acids in the must of the investigated varieties ranges as follows:

- Cabernet Sauvignon – 6.50 – 9.00 g/dm^3;
- Gamza – 5.90 – 8.90 g/dm^3;
- Rubin – 5.50 – 6.00 g/dm^3

The Cabernet Sauvignon’s and Rubin’s musts showed a slightly higher content of titratable acids, while Gamza was characterized by a lower content of this indicator, compared to the suggested by the cited authors. The lower content of titratable acids was explained by the higher sugar accumulation in these varieties, which was reflected in a slight decrease in their titratable acidity. In addition, the content of titratable acids is a highly variable factor depending on variety, geographical area and climate.

pH represents the relationship between the amount and strength of the acids. The pH of the grape must should vary in the range 2.80 – 3.80 (Chobanova, 2012). The must of the control (Cabernet Sauvignon) showed the lowest pH (2.97±0.000). The obtained data for this indicator in the must of all investigated varieties were normal and correlated with those presented by Chobanova (2012).

The results of the three main technological indicators (sugars, TA and pH) indicated that the grapes were harvested and processed at technological maturity.
The phenolic complex is a major factor for the biological value of grapes and wine, determining their antioxidant activities.

In the grape must of the studied varieties, total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds (NPC) and anthocyanins were determined. The obtained results for the phenolics are presented in table 2.

Table 2. Content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds and anthocyanins in grape must of the studied varieties

Grapevine varieties	**TPC g/dm^3**	**FPC mg/dm^3**	**NPC g/dm^3**	**Anthocyanins, g/dm^3**
Cabernet Sauvignon	0.79±0.00	55.09±12.98	48.80±1.58	12.14±0.27
Gamza	0.45±0.00	284.71±4.15	134.40±0.25	3.76±0.50
Rubin	1.18±0.00	1412.05±5.95	177.97±0.37	35.01±0.32

When analyzing the results for TPC content in the must of the studied red varieties, it could be saw that Rubin showed a significantly high content. It exceeded more than twice the content of TPC found in Gamza (the lowest of the three studied varieties) and more than one time that found in the control introduced variety Cabernet Sauvignon. The hybrid red grapevine variety Rubin at the growing conditions of the area showed a good ability to accumulate large amounts of phenolic compounds, which suggested a high biological potential. The data on the determined content of TPC in the studied must of the red variety Cabernet Sauvignon were in absolute correlation with the studies of Franco-Bañuelos *et al.* (2017) who found TPC content of 321.90 mg GAE. 100 g^{-1} to 607.60 mg GAE. 100 g^{-1} in a study of grapes from four red wine varieties (Cabernet Sauvignon, Merlot, Rubired and Petite Syrah) from the region of Mexico. The data in the present study were also correlated with the range of variation (1000.00 – 5000.00 mg/dm^3) of TPC in red grape varieties presented by Velkov (1996).

The analysis of the data showed that the hybrid Rubin had the highest quantitative presence of FPC. Compared to the control introduced variety Cabernet Sauvignon, it was twenty-five times more. Cabernet Sauvignon showed the lowest quantitative value for this indicator. In the must of the local Gamza variety FPC were nearly five times lower than that of the Rubin hybrid.

The Rubin hybrid had the highest content of NPC, followed by the local variety Gamza (the difference between them was not very large). It was found that the concentration presence of NPC in the must of the control introduced variety Cabernet Sauvignon was more than three times lower, compared to the NPC of the hybrid variety Rubin.

Some representatives of the NPC groups exhibit different biological activities (Chobanova, 2012), related to the manifestation of antiseptic properties, bactericidal activity, anticholesterolemic effect.

The content of anthocyanins in fresh grape must (immediately after grapes crushing) of the three studied varieties was also determined. Due to the weaker contact during grapes crushing (as the first phase of technological processing), the amounts of these phenols in the fresh grape must were low. The results showed that the must of the red hybrid variety Rubin was characterized by the highest content of anthocyanins. Their amount exceeded nearly three times that found in the control Cabernet Sauvignon and 11 times that found in the must of the local Gamza variety.

It could be saw from the results for the overall phenolic composition of the must (table 2) from the studied varieties that Rubin excelled not only in the "anthocyanin content" indicator, but also demonstrated significantly higher levels of all studied phenolic compounds groups (TPC, FPC, NPC). The obtained results were evidence for the potential of this variety, in the conditions of the town of Pleven (Central Northern Bulgaria), to accumulate high amounts of phenols, which reflected in a direct probability of the biological benefits manifestation.

The antioxidant activity is one of the main factors determining the biological potential of grapes and wines. The data regarding the antioxidant activity of the must from the studied varieties are presented in table 3.

Table 3. Antioxidant activity (in %) of grape must from the studied varieties

Grapevine varieties	**Antioxidant activity (in %) of grape must with different total extracts and reaction times**			
	Total extract of 600.00 mg/dm^3		**Total extract of 400.00 mg/dm^3**	
	5th min	**15th min**	**5th min**	**15th min**
Cabernet Sauvignon	40.86±0.16	42.29±0.005	41.24±0.22	49.96±0.03
Gamza	44.49±0.21	48.73±0.06	33.70±0.29	42.49±0.09
Rubin	55.79±0.17	54.73±0.02	51.30±0.03	52.17±0.02

The results for the antioxidant activity at TE = 600.00 mg/dm^3 showed that the highest potential for free radicals capturing was found in the must of the red hybrid variety Rubin. After Rubin came Gamza. The lowest antioxidant activity for the same extract was recorded in the must of the control introduced variety Cabernet Sauvignon. Thus, Cabernet Sauvignon must showed significantly lower antioxidant activity than that of the Rubin hybrid. In 5 min of the reaction at TE=600.00 mg/dm^3 it was nearly 15% lower, and in 15 min of the reaction Cabernet Sauvignon showed 12% lower ability to eliminate the free DPPH• radicals.

The obtained data for the antioxidant activity of the must from the studied red varieties, at TE=600.00 mg/dm^3, correlated with the obtained data for the amount of FPC and NPC. The following correlation dependence was obtained: FPC+NPC+AA (hybrid variety)>FPC+NPC+AA (local variety)> FPC+NPC+AA (introduced variety). The two groups of phenolic compounds consist of subgroups with representatives showing significant free radical scavenging potential - catechins, proanthocyanidins, anthocyanins, flavones, flavonones and falvonols (FPC) (Harbone, 1980), phenolic acids, coumarins and stilbenes (NPC) (Chobanova, 2012). The study proved a direct correlation between the two groups of phenolic compounds (FPC and NPC) and the established antioxidant activity in the must of the studied red grapevine varieties grown in the conditions of the town of Pleven, Central Northern Bulgaria.

From the results for the obtained antioxidant activity of the must from the studied red varieties at the lower total extract (400.00 mg/dm^3) Rubin again showed the highest activity recorded. Second by the antioxidant capacity was the must of Cabernet Sauvignon variety. The Gamza must by the studied sample extract (400.00 mg/dm^3) showed the lowest antioxidant activity.

The study related to the antioxidant potential of the must from the studied red varieties in both extracts showed a dominance of Rubin. The hybrid grapevine variety demonstrated high antioxidant activity, potentially exceeding that found in both the control introduced and the local varieties. The relationship was also confirmed through the established correlation between FPC, NPC and the antioxidant activity of the red grapevine varieties.

The grapes were vinified according to the classic scheme for the production of dry red wines (Yankov, 1992). The data on the main chemical indicators of the obtained wines are presented in table 4.

Table 4. Chemical parameters of the experimental wines, harvest2021

Wines	**Alcohol content vol. %**	**Total extract g/dm^3**	**Sugars g/dm^3**	**Titratable acids g/dm^3**	**pH**
Cabernet Sauvignon	13.24±0.08	26.30±0.17	3.35±0.08	6.79±0.11	3.92±0.011
Gamza	15.01±0.10	24.96±0.51	3.53±1.67	5.08±0.14	4.24±0.005
Rubin	14.58±0.07	36.33±0.05	6.57±0.02	6.77±0.11	4.10±0.000

The amount of ethyl alcohol in Gamza was the highest (15.01±0.10 vol. %) but its difference with Rubin (14.58±0.07 vol. %) was very small. The ethanol content in Cabernet Sauvignon wine was the lowest (13.24±0.08 vol. %). Ethyl alcohol normally varies in wines in the range 7.00 vol.% - 17.00 vol.% (Chobanova, 2012).

Almost all analyzed wines showed a normal total extract. The highest extractability was showed by Rubin (36.33±0.05 g/dm^3). The wines of Cabernet Sauvignon (26.30±0.17 g/dm^3) and Gamza (24.96±0.51 g/dm^3) did not show a big difference by this indicator, but a slight preponderance was observed for Cabernet Sauvignon.

According to the established content of residual sugars, Cabernet Sauvignon (3.35±0.08 g/dm^3) and Gamza (3.53±1.67 g/dm^3) belonged to the dry wines category. The Rubin wine (6.57±0.02 g/dm^3) had a higher content, as a result of the higher sugars in the grapes, the accumulation of the maximum amount of alcohol and the incomplete course of the alcoholic fermentation.

An almost similar content of titratable acids between Cabernet Sauvignon (6.79±0.11 g/dm^3) and Rubin (6.77±0.11 g/dm^3) was found. The titratable acids content (5.08±0.14 g/dm^3) was lower in Gamza's wine. All established levels of titratable acids were in the optimum (5.00 to 9.00 g/dm^3) for this indicator (Chobanova, 2012).

Regarding the actual acidity (pH) a variation from 3.92±0.011 (Cabernet Sauvignon) to 4.24±0.005 (Gamza) was found.

The data on the content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds (NPC) and anthocyanins in the studied wines are presented in table 5.

Table 5. Content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds and anthocyanins in wines of the studied varieties

Wines	**TPC g/dm^3**	**FPC mg/dm^3**	**NPC g/dm^3**	**Anthocyanins, g/dm^3**
Cabernet Sauvignon	1.55±0.00	6709.18±5.40	410.51±0.56	303.11±0.16
Gamza	1.05±0.005	3341.01±11.06	417.77±0.66	253.02±5.90
Rubin	4.23±0.005	4223.41±20.90	270.84±0.29	287.26±0.13

The wines showed very significant differences in the concentration of TPC. The content of TPC in the wine of the hybrid variety Rubin was significant and very high. The result was in correlation with the established higher levels of TPC found in its grape must (table 2). Second in quantitative presence of phenols was the wine of the Cabernet Sauvignon variety. The lowest phenolic content was found in Gamza. The data were in correlation with the results of this indicator for the grape must of the studied varieties (table 2). The data regarding the amount of TPC detected correlated with the study of Shahidi and Naczk (1995), in which a variation for the presence of TPC in red wines from 1000 to 4000 mg/dm^3 was found. Li *et al*. (2009) found a range of variation in TPC of studied red wines from 1402.00 to 3130.00 mg/dm^3. Our data were also in agreement with the study of Radeka *et al.* (2022) who investigated red wines from the Croatian varieties Teran and Plavac and found a TPC variation from 1527.12 mg/dm^3 to 3936.21 mg/dm^3. The data also correlated with the results of Nistor *et al*., (2015), who investigated the content of total phenolic compounds in Cabernet Sauvignon and Pinot Noir wines from three consecutive harvests (2011, 2012 and 2013) produced by two different wineries. A variation of 2072.00±81.38–2758.00±149.90 mg/dm^3 (Cabernet Sauvignon) and 1814.00±92.51–2695.00±76.46 mg/dm^3 (Pinot Noir) was found for the established total phenolic compounds from the first group of red wines. In the case of red wines from the second group, the total phenolic content was found within the limits of 1986.00±163.50–2531.00±77.76 mg/dm^3 (Cabernet Sauvignon) and 1752.00±94.90–2214.00±35.56 mg/dm^3 (Pinot Noir) (Nistor *et al*., 2015).

The data on the established flavonoid phenolic compounds (FPC) showed that the control variant - the wine of the introduced variety Cabernet Sauvignon, had the highest content of FPC. Second, according to the concentration presence of this group of compounds, was the wine of the Rubin hybrid, and the lowest FPC concentration was found in the red wine of the local Gamza variety.

Main representatives of the flavonoid group of phenolic compounds in grapes and wines of *Vitis vinifera* L. varieties are anthocyanins, flavan-3-ols, tannins and their reaction products (Casassa, 2017).

Mitrevska *et al.* (2020) investigated commercial Macedonian red and white wines and found that total flavonoid content in red wines ranged from 547.00±10.00 mg/dm^3 to 1732.00±7.00 mg/dm^3. The data in our study related to the content of FPC was higher.

The wine of Gamza variety showed the highest content of NPC, which was very close to that found in the control introduced Cabernet Sauvignon variety. The lowest NPC content for the red wines was found in Rubin. The non-flavonoid phenolic compounds (NPC) present in wines include representatives of phenolic acids and stilbenes (Fernandes *et al.,* 2017). Woraratphoka *et al*. (2007) investigated the content of phenolic compounds in selected wines from northeastern Thailand. The team found a variation of NPC in the studied red wines from 195.30±3.70 mg/dm^3 to 575.60±341.60 mg/dm^3. The data in our study correlated with those established by the cited team.

The anthocyanins are the red pigments of the varieties and are mainly responsible for the wine color. They are available in wines in amounts from 200.00 to 500.00 mg/dm^3 (Chobanova, 2012). In the studied harvest, the highest anthocyanin content was found in the wine of the control introduced variety Cabernet Sauvignon, followed by the wine of Rubin, and their content was the lowest in Gamza. The anthocyanins are part of the FPC group. The established results for FPC as a trend overlaped with the established anthocyanin content, namely in the order: (Cabernet Sauvignon) > (Rubin) > (Gamza). This was direct evidence that anthocyanins had a significant contribution to the formation of the total concentration of flavonoid phenolic compounds. Our results correlated with data of Tsiakkas *et al*. (2020) who found anthocyanin content of red wines from Cyprus ranged from 24.44±2.61 mg/dm^3 to 509.18±8.18 mg/dm^3. Slightly higher levels of anthocyanins were found by Kharadze *et al.* (2018) in a study of wines obtained from Georgian endemic varieties. The team found a variation in the anthocyanin content of the studied wines from 327.10 mg/dm^3 to 871.70 mg/dm^3. The data on the established antioxidant activity in the red wines of the studied varieties are presented in table 6.

Table 6. Antioxidant activity (in %) of wines from the studied varieties

Wines	**Antioxidant activity (in %) of wines with different total extracts and reaction times**			
	Total extract of 600.00 mg/dm^3		**Total extract of 400.00 mg/dm^3**	
	5th min	**15th min**	**5th min**	**15th min**
Cabernet Sauvignon	55.91±0.37	75.31±0.07	24.03±0.47	41.48±0.14
Gamza	62.19±0.54	71.93±0.06	27.69±0.27	33.06±0.08
Rubin	59.60±0.21	60.05±0.22	54.75±0.29	55.21±0.15

At TE = 600.00 mg/dm^3, high values of antioxidant activity in the wines of the Cabernet Sauvignon and Gamza varieties were observed. In 5 min of reaction with this extract, the wine of Gamza variety showed a higher radical-capturing capacity, compared to Cabernet Sauvignon. In 15 min of the reaction, an increase in radical-elimination activity was observed in both varieties, being more significant in the wine of the introduced Cabernet Sauvignon variety, comparing it to the local Gamza.

When the concentration of TE=400.00 mg/dm^3 was reduced, a significant drop in the wines antioxidant activity of both varieties was observed, but the trend for the strength of the activity at 5 and 15 min was preserved. On the other hand, the wine of the hybrid variety Rubin at TE=600.00 mg/dm^3 in 5 min of the reaction showed an antioxidant activity, which was slightly higher than that of the control introduced variety Cabernet Sauvignon and slightly lower than the established for the wine of the local Gamza variety. At 15 min of the reaction with this extract, Rubin showed a lower antioxidant activity than the wines of the other two investigated varieties.

The situation with Rubin based on TE=400.00 mg/dm^3 was interesting. Compared to the previous extract, a decrease in antioxidant activity was observed, but it was much smaller compared to the same in the wines of the other two varieties. At both 5 min and 15 min, Rubin's wine showed higher antioxidant activity, comparing it to the analogous results of the other two varieties. From these results, it could be concluded that Rubin's wine showed a smoother and more stable manifestation of antioxidant activity, regardless of the extract.

The data on the established antioxidant activity in red wines can be closely related to NPC results. Gamza and Cabernet Sauvignon showed a close concentration presence of this group of phenols, which correlated with the close values of antioxidant activity found in the wines of these two varieties.

The data obtained from our study were in correlation with the research of Bajčan *et al.* (2016) who studied 28 Cabernet Sauvignon red wines from different regions of Slovakia and found an average DPPH antioxidant activity in the range 69.00–84.20%. Our data were also in agreement with the research of Banc *et al.* (2020) who studied red wines from different varieties and regions of Romania and found antioxidant activity ranging from 63% to 95%.

CONCLUSIONS

The must of the Rubin variety demonstrated significantly higher levels of all investigated groups of phenolic compounds (TPC, FPC, NPC and anthocyanins) in comparison with the other two investigated grapevine varieties. The obtained results were evidence for the potential of this variety, in the conditions of the town of Pleven (Central Northern Bulgaria), to accumulated high amounts of phenols, which reflected in a direct probability of the manifestation of biological benefits.

The study related to the antioxidant potential of the must in both extracts showed a dominance of Rubin. The hybrid variety demonstrated high antioxidant activity, potentially exceeding that found in both the control introduced and the local varieties. The relationship was also confirmed by the established correlation between FPC, NPC and antioxidant activity.

A high presence of TPC in the analyzed wines was identified in the wine of the Rubin hybrid. It exceeded almost three times that found in the wine of the control introduced variety Cabernet Sauvignon and four times that found in the wine of the local variety Gamza.

Gamza and Cabernet Sauvignon wines showed very close concentrations in their NPC contents, while Rubin showed a lower content.

The established results for FPC in the wines as a trend overlap with the established anthocyanin content, namely in the order: Control introduced variety (Cabernet Sauvignon) > Hybrid variety (Rubin) > Local variety (Gamza). This was direct evidence that anthocyanins have a significant contribution to the formation of the total flavonoid phenolic compounds concentration.

The data on the established antioxidant activity in red wines could be closely related to the NPC results of the wines. Gamza and Cabernet Sauvignon showed a close concentration presence of this group of phenols, which correlated with the close values of antioxidant activity found in the wines of these two varieties.

The study proved high biological potential and good phenolic accumulation in grapes and wines of the studied varieties, a result that was closely related to the characteristics of the Central Northern Bulgaria terroir.

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ELISA DIAGNOSIS OF GRAPEVINE PINOT GRIS VIRUS

SUMMARY

The grapevine production capacity and the products quality depend largely on the health of the cultivated plants. Of the pathogens affecting the grapevine culture, viruses cause significant damages. For this reason, it is extremely important to use diagnostic methods to identify the pathogens and to choose the correct method of plant protection. A reliable method for the routine diagnosis of many economically important grapevine viruses is Enzyme -Linked Immunosorbent Assay (ELISA), a number of kits allowing the detection of different grapevine viral pathogens being commercially available. The increased incidence of *Grapevine Pinot gris virus* (GPGV) in plantations around the world and the availability of an ELISA kit, made it possible to identify this pathogen in Romania as well. The *in-house* validation of GPGV kit was necessary. The results showed that the most reliable type of tissue for analysis is the leaf petiole in the beginning of the growing season and the phloem tissue in the dormancy period. As the ELISA response decreased over the time both for aliquots of positive control of kit and positive plant storage samples, using the plant control is necessary, but carefully for the type of plant tissue and period of sampling.

Keywords: GPGV, validation, cut-off, repeatability, reproducibility, robustness

INTRODUCTION

Historically, visual diagnosis was the first and most used approach to diagnosing grapevine viral diseases, but without identifying the pathogen. Leaf reddening and curling, for instance, can be caused by the *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2) and *Grapevine leafroll-associated virus 3* (GLRaV-3), *Grapevine red blotch-associated virus* (GRBaV) or *Grapevine flavescence dorée* (FD) phytoplasma presence, *flavescence dorée* phytoplasma infection, or the Gra*pevine red blotch-associated virus* (GRBaV). Moreover, the same virus can cause differing

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symptoms in different grapevine varieties. For example, leaf yellowing in white cultivars or reddening in red genotypes can be caused by leafroll-associated viruses. Sometimes, the virus infections have no symptoms. A GLRaV-3 infection in white varieties results in leaf blade yellowing, whereas in red-skinned varieties it causes leaf reddening. Finally, in some cases, a viral infection can be asymptomatic. This is why it is rather difficult to correctly detect and identify a virus on the basis of visual symptoms only, without the use of laboratory testing (Zherdev *et al.,* 2018).

ELISA (enzyme-linked immunosorbent assay) is one of the grapevine viral pathogen detection techniques based on coupling of two reactions of high specificity: antigen (represented by viral protein) – antibody reaction and enzyme – substrate reaction, through a compound called conjugate (Clark and Adams 1977). Currently, a number of ELISA kits allowing for the detection of different grapevine viral pathogens are commercially available.

In ELISA diagnosis, the green parts of the plant (foliar limb, petiole) and the phloem tissue scraping from cane are usually tested. Depending the virus, the season, the development phenophase, the pathogen may be unevenly distributed in different part of the plant. This is the reason why in the diagnostic kit are additional recommendations regarding the sampling period and the tissue types preferred for testing.

The evolution of grapevine viral disease diagnosis like next-generation sequencing method, allows the identification of new pathogens assimilated to characteristic symptoms.

By Illumina high throughput sequencing method, field symptoms of chlorotic mottling and leaf deformation observed since 2003 on the Pinot gris cv., in Italy, have been attributed to a new virus, named Grapevine Pinot gris virus (GPGV), related with trichoviruses (Giampetruzzi *et al.*, 2012). At the meeting of virologists in the frame of the 17th ICVG Congress was announced the discovery of four new grapevine viruses, including GPGV (Martelli, 2012). According to EPPO, updated 21 February 2022, the virus is widespread, and it has been reported in 58 countries on five continents (Demian *et al.,* 2022).

ELISA has been used for the GPGV detection in vineyards and in the certification program both in field-grown and greenhouse-grown grapevines (Tarquini *et al*., 2018; Bertazzon *et al*, 2021).

In Romania, the study about the incidence of GPGV in germplasm collection highlighted that out of 95 samples collected from plants presenting specific symptoms to GPGV infection, 60 were confirmed as infected. On the other hand, out of 75 samples from asymptomatic grapevines, 22 were ELISA positive (Guță and Buciumeanu 2021).

Since a part of our activity is the grapevine viruses monitoring, the reliable use of the ELISA diagnostic method was also studied for *Arabis mosaic virus+Grapevine fanleaf virus* (ArMV+GFLV), GLRaV-1+3 and *Grapevine fleck virus* (GFkV) (Guță and Buciumeanu, 2010, 2011, 2012). Because GPGV is a new virus entered into the study, the aim of this work was to verify the safety of GPGV diagnosis by ELISA reagents.

MATERIAL AND METHODS

ELISA is performed in the grapevine virology laboratory belonging to National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti, Argeş, România, who analyzes the most damaging and widespread grapevine viruses: ArMV+GFLV, GLRaV-1+3, GFkV and *Grapevine virus A* (GVA), both for routine diagnosis and research purposes, using commercial reagents BIOREBA, Switzerland.

Specific equipment (incubator, plate washer, spectrophotometer) and certified NUNC F-96 maxisorp microtiter plates were used.

The results presented in this work were obtained during GPGV testing, over three years, with three diagnostic kits, named A, B, C. The working instructions as recommended by the manufacturer were followed.

Positive and negative control are intended to verify the ELISA performance. Each kit contains one positive control (lyophilized extract of infected plants) and one negative control (lyophilized extract of healthy plants); the expected values of ELISA readings are indicated in the datasheet. For several determinations, the controls are used as aliquots, stored at -20°C. The maintenance in time of the quality of the controls was studied.

The reproducibility intra-determination and the repeatability inter-determination is assessed on positive samples (plant infected extracts) with different optical density (OD) readings, positive and negative controls, stored as aliquots at -20°C. Three repetitions of each extract have been performed for the repeatability. In this case, standard deviation of the mean must be smaller than cut-off. The reproducibility was assessed on aliquots of samples from a grapevine infected plant tested several times.

Because the laboratory has a collection of virus infected grapevine plants for the purpose of use as positive controls, three GPGV infected plants were analyzed during several vegetation phenophases. Three types of plant tissue (limb, petiole, cane) have been used.

The laboratory analyzes a large number of samples both for research purposes and for the maintenance of the collection of viticultural germplasm. Therefore it is necessary sometimes to rationalize the reagents by using them at half volume. In this paper is studied the ELISA method robustness for 100 and 200 μL/well work volume.

The minimum limit of virus detection is the cut-off value. The cut-off value was calculated for each ELISA plate individually, being as three times the mean value of negative control (all values above this cut-off were regarded as positive). Reading were performed with dual filter 405/492 nm.

RESULTS AND DISCUSSION

Viral diseases cause grapevines modifications affecting the quantity, quality of production and longevity of the plantation. Early identification of the infection makes it possible to take measures to limit the damage of virus infection. Diagnostic methods have evolved significantly in recent times, and allow a reliable diagnosis. It is of critical importance to reduce test costs and duration in order to provide wide-scale diagnosis that can be included in comprehensive plant protection plans.

Because in the diagnostic process, positive and negative control are intended to verify the ELISA performance, it is necessary to use every time the aliquots stored at -20°C, even if their OD readings may gradually decrease over time, as indicated in datasheet.

Our study showed a strong variation up and down of positive control after 60 min readings, to all kits, A, B, C, both for 100 (kit A and B) or 200 μL/well (kit C) analysis volume. In all tests OD indicated positive signal, but after eight month (kit A), five month (kit B), two month (kit C), the value was closely of the cut-off (Figure 1, 2, 3). Unexpected, using the aliquot from kit A on the validation of kit B, OD reading increased, although 12 months had passed since solubilization (Fig. 1, point 12). The positive control of the kit C (currently in use) had a similar behaviour, even if 200 μL/well volume was used (Figure 3).

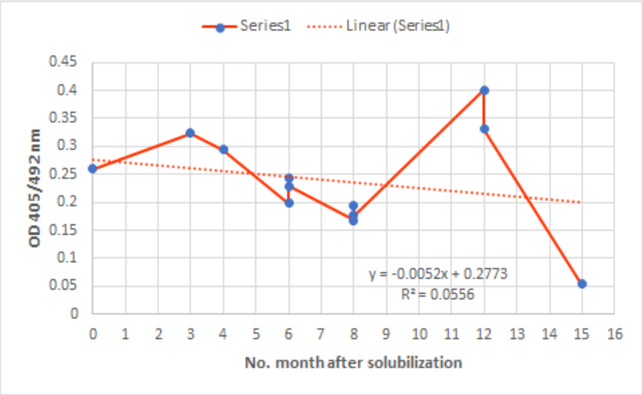

Figure 1. Evolution of ELISA optical density (OD) readings at 405/492 nm of the positive control from Kit A (series 1), used in different tests as aliquots, after solubilisation, for the working volume of 100 μL/well

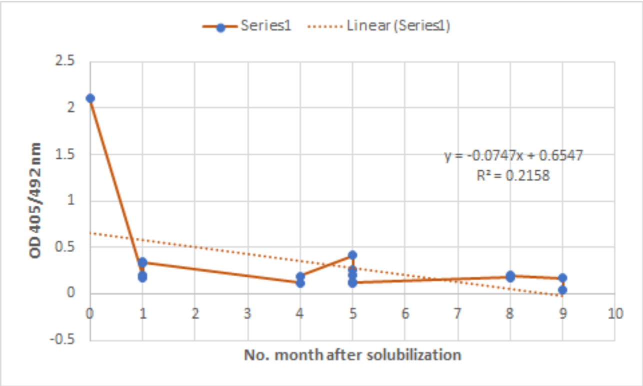

Figure 2. Evolution of ELISA optical density (OD) readings at 405/492 nm of the positive control from Kit B (series 1), used in different tests as aliquots, after solubilisation, for the working volume of 100 μL/well

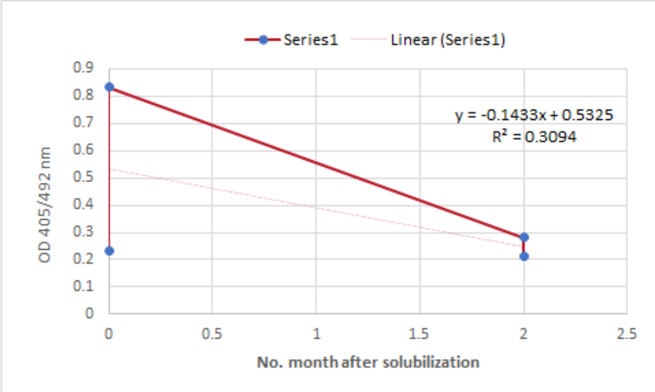

Figure 3. Evolution of some ELISA optical density (OD) readings at 405/492 nm of the positive control from Kit C, used in different tests as aliquots, after solubilisation, for the working volume of 200 µL/well

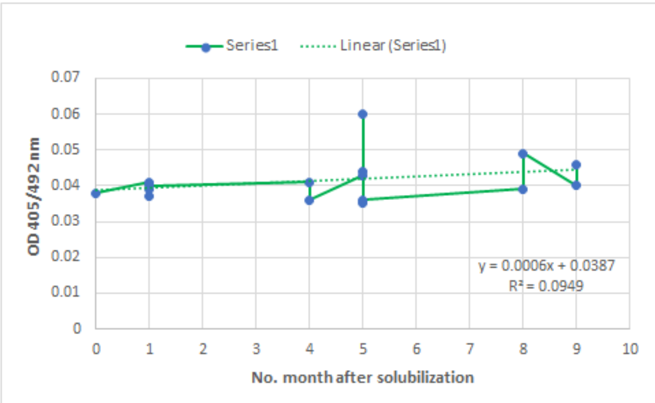

Figure 4. Evolution of ELISA optical density (OD) readings at 405/492 nm of the negative control from Kit B (series 1), used in different tests as aliquots, after solubilisation, for the working volume of 100 µL/well

Also, OD readings at 60 min of negative controls (lyophilized extracts from healthy grapevine plants) have not decreased linearly over time (Figure 4). The study of repeatability of the OD readings of different two samples, positive and negative control showed the precision intra-determination of diagnostic kit (Table 1).

On the other hand, repeated testing of different aliquots samples, both foliar limb or phloem tissue have highlighted decrease over time of 60 min OD readings. In some cases, after several month, the ELISA response was negative (Table 2 and 3).

The robustness of ELISA diagnostic kit for GPGV identification was investigated too, on the validation of kit C comparatively with kit B. OD at 60 min. reading of positive control C after reconstitution was much smaller than extinction values recommended in data sheet (>1.400) with kit C components, while the results were negative with kit B (still in the operating period).

Surprisingly, a positive control A had a positive response 22 months after reconstitution. A positive phloem tissue sample had a negative response 10 months after extraction (Table 4).

Table 1 Repeatability intra-determination: ELISA readings (OD 405/492 nm) for three repetitions of two phloemic tissue samples, positive kit control A and negative kit control A (100 µL working volume)

Repetition	ELISA readings (OD 405/492 nm)			
	Sample 1	Sample 2	Positive kit control	Negative kit control
1	0.522	0.879	0.260	0.054
2	0.510	0.886	0.254	0.046
3	0.512	0.815	0.256	0.047
Mean ± std	*0.514 ± 0.006*	*0.860 ±0.039*	*0.256 ± 0.003*	*0.049 ± 0.004*

cut-off = 0.147

Table 2 Reproducibility inter-determination: ELISA readings (OD 405/492 nm) for some repetition of positive samples (Kit A; 100 µL working volume)

Repetition	Sample 1*	Sample 2*	Sample 3**	Sample 4**	Sample 5*
1	0.709	0.527	0.514	0.860	0.308
2	0.479	0.533	0.151	0.149	0.294
3	-	-	-	0.158	0.174

** foliar limb*
*** phloem tissue sample*

Table 3 Repetability inter-determination: ELISA OD results (OD 405/492 nm) for some repetition of positive samples (Kit B; 100 µL working volume)

Repetition	Sample 1	Sample2	Sample 3	Sample 4	Sample 5
1	1.384	0.138	0.514*	0.860*	0.722
2	0.515	0.079**	0.082**	0.096**	0.098**
3	0.134	0.054**	-	-	-

** OD from the first diagnosis, Kit A, phloemic tissue sample, 16 month ago*
*** negative result of the test*

Technical sheet recommends GPGV diagnosis from young leaves at the top, or middle of the plant, sampled in the spring. The results obtained in ELISA tend to indicate that there is a heterogeneous distribution of the virus from one leaf level to another and that the sampling period is important to consider in the spring (Guide d'information sur l'émergence du virus du Pinot gris et sa propagation, 2019). To verify the ELISA performance, the manufacturer suggests also the use of the plant control.

Table 4 Validation of ELISA Kit C

Sample	Kit B		Kit C	
	100 µL/well cut off = 0.117	200 µL well cut off = 0.201	100 µL/well cut off = 0.114	200 µL well cut off = 0.204
Positive control C at solubilization	0.073	0.073	0.311*	0.232*
Positive control B – aliquot after 10 months from solubilization	0.037	0.069	0.096	0.146
Pozitive control A - aliquot after 22 months from solubilization	-	0.063	0.127*	0.222*
Positive floemic tissue (OD = 0.136, ten months before)	0.037	0.067	0.053	0.089
Positive floemic tissue (OD = 0.860, ten months before)	-	-	-	0.166
Positive floemic tissue (OD = 0.514, ten months before)	-	-	0.090	-

** the positive responses*

In our study, in April, all the samples taken from the leaves (limb and petiole) at the base or the top of the shoot, were negative. Thermal amplitude (42°C average) and the maximum temperatures (43°C average) reached in the greenhouse during the sample period, may be the cause of these results.

At the beginning of June, after flowering, petiole samples were positive, while the limb readings showed negative results, along the length of the shoots (Figure 5).

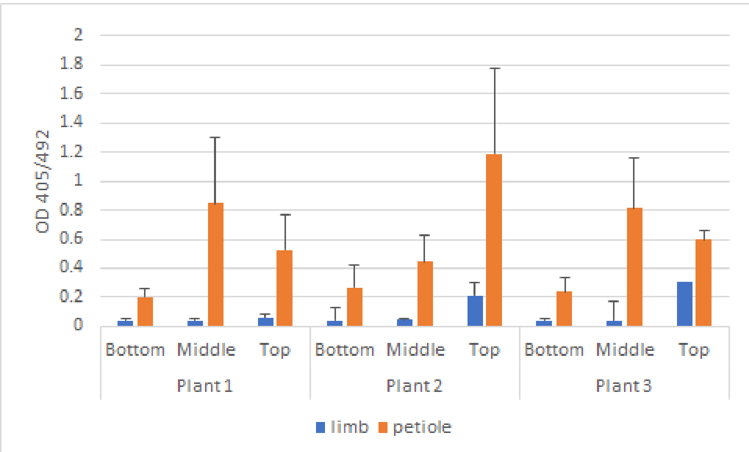

Figure 5 ELISA diagnosis of GPGV from leaves (limb and petiole) collected from the bottom, middle and top of the shoots of three infected plants. Values are average from three repetition (3 shoots/plant), bars represent the std.

In this phenophase of vegetation, although the maximum temperatures recorded were around 42°C, the thermal amplitude decreased to 27°C in average. In this condition, the petiole as conductive tissue was the best material to sample for a correct result.

As expected, the analysis of the phloemic tissue, along the length of the canes, during the dormancy period confirmed the GPGV infection of plants.
Grapevine (*Vitis vinifera* L.) is an important crop who is affected over of 80 distinct number of viruses (Martelli, 2017), some of them with important economic impact, requiring early identification to limit damages. That is why it is necessary to constantly develop new diagnostic methods with high detection sensitivity at an affordable cost price.

ELISA is a robust method with a high detection sensitivity, 1 to 10 ng virus/ml. Eight from fourteen specific grapevine viruses, among which the GPGV, have the possibility of identification through ELISA method.

Diagnostic methods based on nucleic acids amplification are more sensitive but requires expensive equipment and reagents.

There were only few studies that comparatively investigated the serological and molecular methods of grapevine virus diagnosis. When using phloem tissue as a biological material for analysis, some researchers indicated an equal reliability of ELISA and RT-PCR (Ling *et al*., 2001; Fiore *et al*., 2009). Chen *et al.* (2003) indicated that RT-PCR was more reliable for the detection of GLRaV-3 than ELISA while other studies showed that ELISA may be more sensitive than RT-PCR for the detection of GLRaV-1 and -3 (Cohen *et al.*, 2003). ELISA identification of GLRaV-1, -2 and -3, GVA, GFkV and GFLV from leaf petioles was more sensitive than RT-PCR as compared to the use of leaf blade and phloem tissue during the summer and early fall (Fiore *et al*., 2009).

The possibility of the simultaneous detection of a large number of viruses by one assay using DNA chips is essential for the diagnosis of grapevine infections. Since mixed infections with several pathogens at a time is an often-seen in vineyards, this feature of the DNA chip technique is a significant advantage not only for research purposes, but for further commercialization.

CONCLUSIONS

The results obtained on GPGV diagnosis by ELISA using BIOREBA commercial kit, showed that the most reliable type of tissue for analysis is the leaf petiole at the beginning of the growing season and the phloemic tissue in the dormancy period.

ELISA response decreased over the time both for kit positive aliquots control and positive plant storage samples.

Using the plant control is necessary, but carefully for the type and time of sampling. Compliance with work volume/well as manufacturer recommended, eliminated any doubts on the results obtained.

The presence in the GPGV detection kit of several controls, without dividing the solubilized control into small volumes (aliquots), would increase the diagnosis reliability.

ELISA remains a reliable method for the routine diagnosis of many economically important grapevine viruses. Although virus titre shows seasonal fluctuations and the viruses may be unevenly distributed in grapevines, particularly for recent infection, ELISA provides reliable diagnosis if samples are collected at the optimal time in the specified grapevine tissue. ELISA results should be supplemented by molecular tests in critical situations, since some viral strains may be not detected by one or other type of tests.

ACKNOWLEDGEMENTS

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COMPARATIVE ASSESSMENT OF SELECTED EXOTIC HYBRID RICE (*Oryza sativa* L.) BASED ON QUANTITATIVE TRAITS ANALYSIS

SUMMARY

In the plant breeding program, significance and nature of genetic diversity plays a pivotal role in identifying a suitable cultivar for its further improvement. The present study was conducted to compare and analyse the contributions of yield components to grain yield of twenty-one exotic hybrid rice genotypes under field conditions, and evaluate the relationships between 11 grain yield and its components of hybrid rice to identify the suitable cultivars. The principal component analysis showed that the first three components were significant with eigenvalue more than 1 and contributed 50.74%, 16.32% and 11.14% of the total variance, respectively. PC1 included the traits that were related mostly to the plant height (cm), effective tillers, panicle length (cm), grain width (cm), 1000 grain weight (g) and grain yield (t ha^{-1}). Cultivars from Heera-2, Qyou-6, SHD-661, Q-5, Q-28, HS-366 performed well in PC1 while cultivars such as HS-558B, T-35, Xiang-11, Lyongyou-5, RXEL-16, RXME-22 performed well in PC2. The cluster analysis grouped the cultivars into three main clusters in which 14 cultivars were grouped into cluster I. And cluster I showed the highest mean value for plant height, panicle length, filled grain with moderate high rate of

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effective tillers which were directly influenced to produce higher grain yield. Therefore, the Chinese cultivars SHD-661, Qyou-6, Q-5 and Q-28 from the clusters I can be used in the further development of the superior hybrids for commercial cultivation in Bangladesh.

Keywords: hybrid rice, quantitative traits, multivariate analysis, yield

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major and staple food crops, feeding more than half of the global population (USDA, 2022). Asia is the key source of rice genetic diversity in which almost 90% of the world's rice is grown and consumed, where almost 50% people in Asian countries rely on rice grain (Tenorio *et al.,* 2013). In Bangladesh, 11.4 million hectares land area was involved in rice cultivation, generating 51.6 million tons of rice annually. And 77% of the total cropped area was used for its production which contributed more than 80% of the country's food supply (BBS, 2022). Presently, rice singly constitutes almost 93% of the country's total food supply, achieving 75% of the caloric intake along with 50% of total protein intake in the country (Pervez, 2018). But over-increasing population growth rate in Bangladesh is a great threat to its food security, lowering the cultivable land area due to the urbanization and industrialization. And the rice production needs to be increased by at least 60% to fulfil its food requirement of such population by the year 2030 (Masum, 2019). Regarding this issue, increasing the rice yield per unit land area is one of the best possible ways to meet the food demand even in ever-increasing trend of population in Bangladesh.

Hybrid rice has the capability to increase almost 20-30% more yield than high yielding varieties (HYV) and traditional rice varieties (Fazal *et al*., 2022; Hossain *et al*., 2017; Yesmin *et al.,* 2022). In the last two decades, much emphasis has been given to cultivate hybrid rice for the improvement rice production in Bangladesh (Azad *et al*., 2022; Hasan *et al*., 2022). The 0.7 million hectares land were cultivated hybrid rice during Boro season in 2016-17 which has contributed 3-4 million tons of additional rice to the total rice production of the country (AIS, 2018). Although there are few risks of hybrid production in Bangladesh (Kanak Pervez *et al*., 2022), in the meantime by adopting new technology, more than a dozen of hybrid rice varieties have been developed (Rahman *et al.,* 2019; Rabbi *et al*., 2020). For the time being, effective seed production infrastructure is developed in the government, private, or NGO sectors in Bangladesh those are closely linked with hybrid rice research to accelerate the higher yield production. And the government encouraged the private seed companies to import hybrid seeds from abroad which can boost up the production at farmers level through on-farm trials in different locations of Bangladesh (Julfiquar *et al*., 2006; Shah *et al.,* 2014). So, the present study was emphasized the hybrid rice varieties in the farmer's field those were collected from China and India.

Multivariate traits analysis is one of the useful statistical tools which are broadly used to summarize and describe the inherent variations among the cultivars. Principal component analysis (PCA) is used to identify the similar set of accessions which can captures the maximum genetic diversity (Gireesh *et al.*, 2017) and eliminates the redundancy in datasets, as variation occurs commonly found in plants for yield and its attributing traits (Mahendran *et al.*, 2015). And correlation analysis determines the association between yield and its related traits (Oladosu *et al.*, 2018) and also brings out the relative significance of their direct and indirect effects which could help to the breeders for choosing the selection strategies (Rahman *et al*., 2022). A high yield potential with short duration rice varieties is a common objective of a rice breeder (Hasan *et al*., 2008; Hossain *et al*., 2010; Huang *et al*., 2015). Therefore, genetic investigation through multivariate traits analysis could help to select superior rice genotypes.

Variety itself plays an influential role for the improvement of a specific crop even in a distinct character. And effective utilization of the available genetic diversity exists in rice varieties can helps for its improvement strategies (Sarkar *et al*., 2021; Tabassum *et al*., 2020a; Tabassum *et al*., 2020b). In the meantime, the government of Bangladesh as well as private research organizations has imported some hybrid rice varieties from China, India and Philippines for enhancing the total yield. The wide diversity of such exotic rice could act as a gene pool for the advancement of varietal improvement in Bangladesh. Although, there is available research information on BRRI (Bangladesh Rice Research Institute) derived rice varieties is available, but there is little or no research report on comparative study of exotic hybrid rice varieties in Bangladeshi environment were documented. Thus, the present experiment was investigated to carry out with the objective of studying the genetic diversity, variability and trait relationship associations among the Chinese and Indian hybrid rice varieties based on multivariate analysis.

MATERIAL AND METHODS

Experimental materials and site

In the present study, twenty-one exotic hybrid rice genotypes were grown at the farmer's field in Muktagacha, Mymensingh, Bangladesh (Figure 1) to study their morphological, yield and yield attributing traits. Geographically, the experimental site is situated at 24°25"N latitude and 90°50"E longitude along with medium-high land properties having silty clay loam soil. Among the studied varieties, five (APH-9696, RXEL-16, RXME-22, RXME-23 and TH-35) were collected from India, fifteen (Q-5, Q-28, SHD-85, SHD-422, SHD-557, SHD-661, SHD-726, HS-11, HS-287, HS-366, HS-558B, HS-600, Xiang-11, Qyou-6 and Lyong you-5) were from China and the check variety (Heera-2) which was introduced earlier from China to Bangladesh. The plants were grown in a paddy field during December 2016 to May 2017. The experimental field was medium high land with soil pH of 6.0. Soil contained 2.2% organic matter, 0.15% total nitrogen, 3.25 ppm available phosphorus, 4.48 ppm available sulphur and 0.12

ppm exchangeable potassium. The climate and soil of the study area was under subtropical climate with well drained and non-calcareous grey floodplain fertile soils.

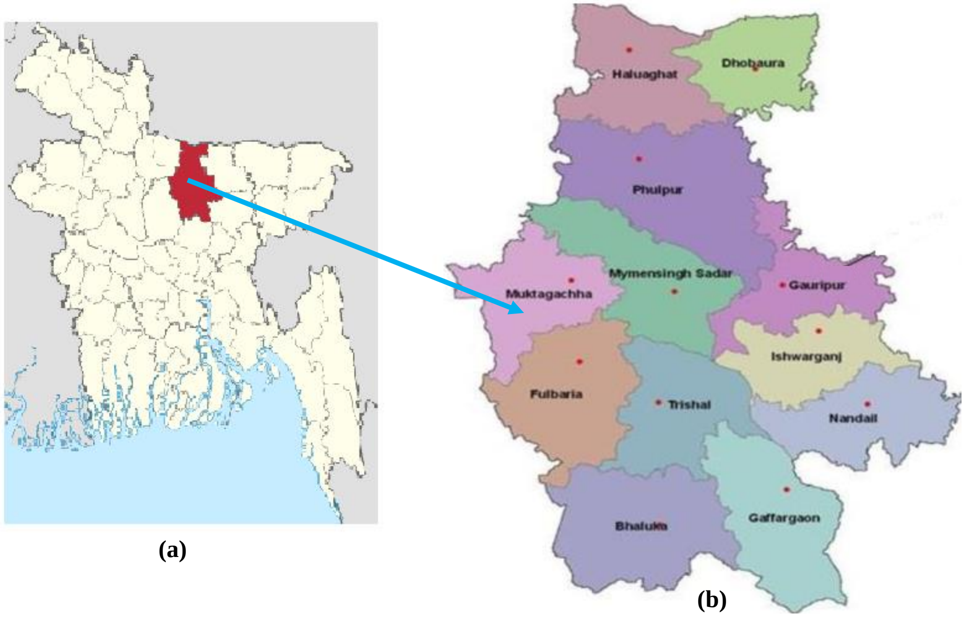


Figure 1. Location of the study area; (a) Bangladesh map showing Mymensingh district (red colour), (b) Muktagacha upazila under Mymensingh district (purple colour)

Experimental design

Single factor experiment was conducted using twenty hybrid rice varieties along with a check variety Heera-2 which were considered as the treatment in the present study. Thirty-five days old single seedling was transplanted per hill for each genotype in randomized complete block design with three replications. Space within and between rows were 20 and 25 cm, respectively and each plot size was 3×2 m= 6 m^2. The fertilizer dose of 280-150-130-70-10 kg per hectare was applied in the form of urea, triple super phosphate, muriate of potash, gypsum and zinc sulphate, respectively. Total fertilizers except urea were applied before the final land preparation. Urea was applied in three instalments at 15, 30 and 45 days after transplanting (DAT). Appropriate control measures were taken against pests and diseases when necessary.

Collection of experimental data

Data were collected randomly from 5 selected plants per plot. An area of 1 meter square was selected in the middle portion of each plot to record the yield of grain and straw. The number of effective tillers was counted based on per meter square area of experimental plot. Other yield parameters viz. plant height (cm), panicle length (cm), unfilled grain, filled grain, grain length (cm), grain width (cm), days to maturity were collected just before the harvesting time of rice plant. The 1000 grain weight (g) and grain yield (initially grain yield was measured kg per plot which was then converted in ton per hectare) were measured after the harvesting of rice plant along with proper sun drying of grain and straw.

Statistical analysis

Five plants per plot were collected and the mean data points were used for statistical analysis. The genotypic effects and pair-wise multiple comparison of treatment means were tested by the analysis of variance (ANOVA) and Tukey Post Hoc (Tukey-HSD) test using R's built-in and Agricolae packages (Mendiburu, 2015). The Multivariate analysis with the Pearson correlation coefficients were calculated by using the software Statistical Tool for Agricultural Research (STAR 2.0.1) (IRRI, 2021). Cluster analysis was performed using Ward's hierarchical algorithm based on squared Euclidian distance statistics. Principle component analysis, eigenvalues, eigenvectors, and 2D biplots were built to complement cluster analysis.

RESULTS AND DISCUSSION

Performance evaluation for quantitative traits

The analysis of variance for the studied traits showed significant differences ($p<0.001$) among the hybrid cultivars (Table 1) which is an indication of inherent genetic diversity of the rice genotypes. In this study, Indian cultivar APH-9696 produced the tallest plant (112.45 cm) while Chinese cultivar HS-287 (75.82 cm) produced the shortest plant among the hybrid rice cultivars. Amidst the yield contributing characters, tillering capacity of rice genotypes significantly influences the production of panicles as well as grain yield (Ye *et al*., 2022). In our study, the significant differences were noticed in the number of effective tillers among the cultivars. The highest mean number of tillers per plant was recorded in HS-366 (8.93) followed by Qyou-6 (8.70) and SHD-661 (8.57). The lowest mean number of tillers per plant was observed in Q-28 (6.70) which was followed by TH-35 (7.00) (12.77). Singh and Mishra (2022) and Hasan *et al.* (2022) also indicated that the yield potentiality of rice plants is depend on their efficient or productive tillers. Moreover, the tillering capacity of rice is influenced by the robust stems and deep root system with improved sink size and lodging resistance features (Gong *et al.,* 2023).

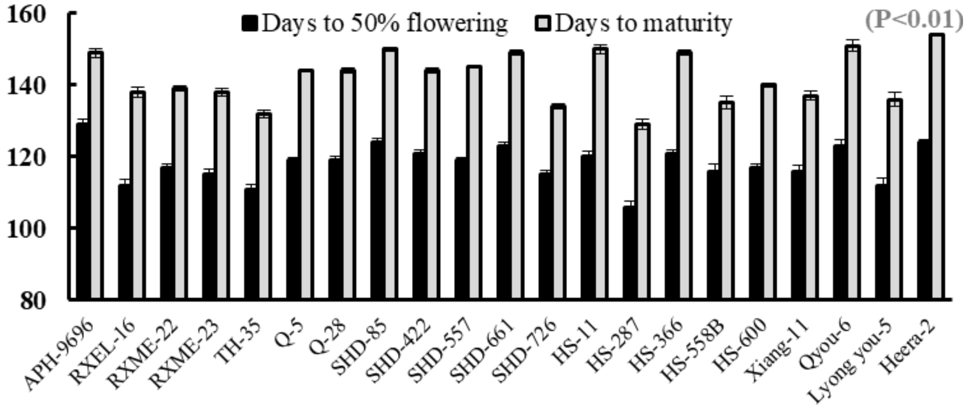


Figure 2. Days to 50% flowering and maturity of hybrid rice varieties

In this investigation, the genotype APH-9696 needed more days for 50% flowering (129 days) while HS-287 was the earliest flowering (106 days) cultivar (Figure 2 and Table 1). In our previous work, we noted the wide variation in days to 50% flowering in the studied cultivars because of the influence of genetic inheritance (Hossain *et al.,* 2017). In addition, Hossain *et al.* (2022) obtained the significant variations in the flowering dates of exotic hybrid rice genotypes grown in Bangladesh throughout the Boro season. In case of maturity, the genotype HS-287 needed the shortest time (129 days) while Heera-2 needed the longest time to mature (154 days). Here, the early heading lines matured earlier and late heading lines matured lately. Yesmin *et al*. (2022) stated that medium maturing genotypes produced better yield as compared to late genotypes in Aman rice in Bangladesh. Among the quantitative traits, panicle length is also an important aspect for improving grain yield in rice. The Chinese cultivar SHD-661 had the maximum panicle length (25.53 cm) followed by Q-28 (24.71 cm) and Qyou-6 (24.54 cm) (Table 1). Panicle length together with spikelet number and seed set determines the grain number per panicle, hence, yield increases in rice (Zheng *et al.,* 2022). The number of filled grain plant^{-1} was the highest in SHD-661 (158.34) followed by Qyou-6 (147.53), HS-366 (135.38) and Heera-2 (146.10). Interestingly, the same cultivars Qyou-6 (14.82), Heera-2 (15.07), SHD-661 (27.93) and HS-366 (28.24) produced the lowest number of unfilled grain plant^{-1}. Kumar *et al.* (2020) demonstrated that the grain yield is directly influenced by the number of filled and unfilled grains per panicle. They found the positive correlation (0.62) for filled grain number and negative and significant correlation for the unfilled grain number with grain yield (-0.47*). Therefore, the grain yield is increased by increasing the number of filled grains and decreasing the number of empty grains per panicle which was noticed earlier by Parida *et al.* (2022) and Fazal *et al.* (2022). Not only the panicle length but also the filled grain number and 1000 grain weight also important determining traits for improving grain yield. Moreover, Qyou-6 showed the highest 1000 grain weight (30.67 g) followed by SHD-661 (26.69) and Heera-2 (26.65) among the tested varieties. This difference might be due to weight and size of the grain. Bai *et al.* (2023) also stated that the weight of rice grain increases with bigger grain size.

In the Table 1, grain yield was markedly differed ($p < 0.001$) among the studied rice varieties. The Chinese hybrid rice SHD-661, Qyou-6 and check variety Heera-2 gave the highest grain yield valued of 10.45 t ha^{-1}, 10.39 t ha^{-1} and 10.29 t ha^{-1}, respectively. These differences happened may be due to the maximum number of effective tillers, panicle length, filled grains and 1000 grain weight. Moreover, regarding the grain yield, Chinese cultivars performed better compared to the Indian as well as check variety due to the higher number of filled grains, effective tillers and 1000 grain weight. Azad *et al*., (2022) and Anwar *et al*. (2022) reported that greater numbers of effective tillers hill^{-1}, filled grains panicle^{-1}, and 1000 grain weight were associated with higher grain yields in hybrid rice.

Association among the quantitative traits

Correlations coefficients measure the strength of association between variables and to determine correlated responses in selection activities. Thus, Pearson correlation analysis was employed in the study to identify the positive or negative correlation among the yield and yield contributing traits to the yield of twenty-one hybrid rice (Table 2). We found that the number of filled grain and the thousand grain weight (g) had a correlation value of 0.82. The number of filled grains produced the more grain weight. Days to flowering had positively significant association with days to maturity. In addition, panicle length (cm) had positive correlation with plant height (cm) and grain yield (t ha^{-1}) but not significantly differ with former to the latter. On the other hand, days to flowering showed significant negative association with panicle length (cm). Moreover, we found a large increase in the number of unfilled grains influenced a decreasing trend in the rice grain yield (t ha^{-1}). Days to flowering had positively significant association with days to maturity, grain width and 1000 grain weight but significant negative association with panicle length (Hasan-Ud-Daula and Sarker 2020; Kumar *et al*., 2018; Rahman *et al*., 2022). The yield component, a quantitative attribute, has a significant impact on grain yield (Kumar *et al*., 2021). The entire effect of quantitative traits on grain yield, either directly or indirectly caused by environmental factors, genetic factors, and their interactions, is one way to quantify the correlation coefficient (Faysal *et al.*, 2022).

Principal component analysis

Multivariate analysis technique was used to assess magnitude of genetic variation in the Chinese and Indian hybrid rice varieties. Figures 3 and 4 showed that the eigenvalue and contribution of each principal component to the total explained variance in the phenotypic diversity of rice, respectively. It was found that three of the eleven principal components had eigenvalue more than 1 and contributed to 78.19% of the variance. PC1 accounted for the highest variance (50.74%) and traits that positively contributed to variation included plant height (0.35), panicle length (0.32), filled grain (0.38), grain width (0.01), days to 50% flowering (0.38), days to maturity (0.38), thousand grain weight (0.37) and yield (0.39) that were mainly related to grain yield, yield attributing, and grain characteristics. PC2 contributed 16.32% of the total variation where plant height (0.24), panicle length (0.23), grain length (0.37) and grain width (0.58) contributed in a positive way. The PC3 accounted for 11.14% variance included plant height (0.05), panicle length (0.20), unfilled grain (0.55), filled grain (0.06), grain width (0.05), days to 50% flowering (0.16) and days to maturity (0.11) (Table 3). Principal components with eigenvalues>1 suggest that the corresponding component explains more variance than a single observed variable (Greenacre *et al.,* 2022; Shrestha *et al*., 2021). Similarly, in an assessment of physico-chemical and cooking characteristics of rice, Pokhrel *et al*. (2020) reported that the first four principal components with vector values>1 retained 73.8% of the total variance.

Table 1. Performance of hybrid rice cultivars for different quantitative characters

Traits	Plant Height (cm)	Effective Tiller	Panicle length (cm)	Unfilled grain	Filled grain	Grain length (cm)	Grain Width (cm)	Days to 50% flowering	Days to maturity	1000 grain weight (g)	Yield (ton/ha)
APH-9696	112.45^{a}	7.83^{a-e}	23.70^{b-d}	66.31^{ab}	107.54^{d-g}	7.20^{m}	1.93^{k}	129^{a}	149^{a-c}	24.10^{d}	9.07^{fg}
RXEL-16	100.44^{e-h}	7.53^{b-e}	22.26^{fg}	48.76^{b-e}	79.39^{hi}	9.20^{ef}	2.47^{h}	112^{ef}	138^{e-g}	21.11^{f}	7.77^{i}
RXME-22	104.38^{b-e}	8.47^{a-c}	22.20^{fg}	42.19^{d-f}	95.24^{f-h}	8.80^{h}	2.87^{bc}	117^{c-e}	139^{d-f}	23.33^{de}	7.80^{i}
RXME-23	94.03^{j}	7.63^{a-e}	22.93^{d-f}	59.99^{b-d}	94.34^{f-h}	8.73^{hi}	2.43^{hi}	115^{d-f}	138^{e-g}	16.57^{g}	7.16^{j}
TH-35	97.13^{h-j}	7.00^{de}	22.60^{ef}	29.36^{e-g}	96.98^{e-h}	9.10^{fg}	2.63^{ef}	111^{fg}	132^{gh}	21.28^{f}	7.93^{i}
Q-5	105.40^{b-d}	7.33^{c-e}	24.09^{bc}	31.18^{e-g}	120.41^{c-f}	10.10^{a}	2.67^{ef}	119^{b-d}	144^{c-e}	23.46^{de}	8.46^{h}
Q-28	98.84^{f-i}	6.70^{e}	24.71^{ab}	33.54^{e-g}	135.68^{a-c}	8.83^{h}	2.70^{d-f}	119^{b-d}	144^{c-e}	25.68^{bc}	9.60^{de}
SHD-85	107.58^{b}	8.13^{a-d}	23.49^{c-e}	63.74^{bc}	126.33^{b-d}	9.10^{fg}	2.47^{h}	124^{ab}	150^{a-c}	24.62^{c}	9.68^{cde}
SHD-422	102.35^{c-f}	8.20^{a-d}	24.02^{bc}	28.95^{e-g}	122.89^{b-e}	7.80^{l}	2.73^{de}	121^{bc}	144^{c-e}	26.35^{b}	10.04^{bc}
SHD-557	101.64^{d-g}	7.50^{b-e}	24.38^{bc}	32.66^{e-g}	88.66^{g-i}	9.80^{b}	2.50^{gh}	119^{b-d}	145^{b-d}	23.53^{cd}	8.71^{gh}
SHD-661	98.71^{f-i}	8.57^{a-c}	25.53^{a}	27.93^{fg}	158.34^{a}	8.27^{j}	3.07^{a}	123^{b}	149^{a-c}	26.69^{b}	10.45^{a}
SHD-726	95.06^{ij}	7.97^{a-e}	23.64^{c-e}	19.79^{g}	98.60^{e-h}	8.87^{h}	2.10^{j}	115^{d-f}	134^{f-h}	24.11^{cd}	9.41^{ef}
HS-11	96.58^{h-j}	7.07^{de}	21.23^{gh}	26.15^{fg}	134.04^{a-d}	8.03^{k}	2.33^{i}	120^{b-d}	150^{a-c}	24.41^{cd}	9.77^{cde}
HS-287	75.82^{k}	8.40^{a-c}	17.31^{j}	65.61^{bc}	50.50^{jk}	9.27^{e}	2.50^{gh}	106^{g}	129^{h}	13.37^{h}	4.49^{l}
HS-366	107.91^{b}	8.93^{a}	23.53^{c-e}	28.24^{e-g}	135.38^{a-c}	8.77^{h}	2.47^{h}	121^{bc}	149^{a-c}	25.82^{bc}	9.84^{cd}
HS-558B	93.17^{j}	7.50^{b-e}	21.36^{gh}	12.83^{g}	67.62^{ij}	9.43^{d}	2.80^{cd}	116^{c-e}	135^{f-h}	13.42^{h}	6.66^{k}
HS-600	105.13^{b-d}	8.57^{a-c}	19.29^{i}	45.34^{c-f}	108.17^{d-g}	9.03^{g}	2.60^{fg}	117^{c-e}	140^{d-f}	23.61^{de}	8.92^{g}
Xiang-11	98.19^{g-i}	7.67^{a-e}	20.99^{h}	14.82^{g}	83.01^{g-i}	9.60^{c}	2.60^{fg}	116^{c-e}	137^{fg}	16.60^{g}	7.63^{i}
Qyou-6	102.40^{c-f}	8.70^{ab}	24.54^{a-c}	20.67^{g}	147.53^{ab}	9.63^{c}	2.60^{fg}	123^{b}	151^{ab}	30.67^{a}	10.39^{ab}
Lyongyou-5	95.02^{ij}	7.60^{b-e}	21.91^{f-h}	86.38^{a}	25.22^{k}	8.60^{i}	2.70^{d-f}	112^{ef}	136^{fg}	11.47^{i}	3.45^{m}
Heera-2	105.80^{bc}	7.37^{c-e}	24.37^{bc}	15.07^{g}	146.10^{a-c}	7.97^{k}	2.93^{b}	124^{ab}	154^{a}	26.65^{b}	10.29^{ab}
Level of sig.	***	***	***	***	***	***	***	***	***	***	***
CV (%)	2.52	10.24	2.83	32.97	15.26	1.07	3.02	2.73	2.76	2.65	5.26

*Means with the same letters within the same column do not differ significantly. *** Significant at the 0.001 probability level*

Table 2. Pearson correlation coefficients among the yield and yield contributing traits of twenty-one hybrid rice genotypes

Traits	Plant Height (cm)	Effective Tiller	Panicle length (cm)	Unfilled grain	Filled grain	Grain length (cm)	Grain Width (cm)	Days to 50% flowering	Days to maturity	1000 grain wt. (g)
Effective Tiller	-0.20**									
Panicle length (cm)	0.70^{ns}	-0.36**								
Unfilled grain	-0.31^{ns}	0.09*	-0.30**							
Filled grain	0.57**	-0.16^{ns}	0.57*	-0.33^{ns}						
Grain length (cm)	-0.12^{ns}	-0.06^{ns}	-0.18**	-0.19**	-0.48**					
Grain Width (cm)	0.29*	-0.34**	0.13^{ns}	-0.28**	-0.10**	0.17**				
Days to 50% flowering	0.68**	-0.03^{ns}	0.66*	-0.21*	0.81*	-0.45**	-0.06^{ns}			
Days to maturity	0.67^{ns}	-0.05**	0.62^{ns}	-0.21^{ns}	0.77**	-0.36*	0.06**	0.90**		
1000 grain wt. (g)	0.62*	0.20^{ns}	0.53*	-0.37**	0.82^{ns}	-0.30**	-0.11^{ns}	0.68^{ns}	0.75**	
Yield (t ha^{-1})	0.66**	0.08*	0.59^{ns}	-0.54**	0.86**	-0.24*	-0.08**	0.75**	0.74*	0.94**

*** Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, ns is non-significant*

The magnitude and direction of contribution of different traits in the different principal components are shown in Figure 5. The PC scores of the genotypes separated them from each other due to variability in the traits along PC1 and PC2. Loading plot identified that the PC1 had the largest amount of varietal variation due to the greater coefficient of plant height, days to 50% flowering, panicle length, filled grain, grain width, days to maturity, thousand grain weight and yield compared to the lower coefficient of unfilled grain and tiller number. The Chinese genotypes SHD-661, Q-5, Q-28, HS-366, Qyou-6 and Heera-2 showed good performance in PC1. Baloch *et al.* (2016) illustrated the positive loadings for days to flowering, panicle length, number of grains panicle-1 and plant height in the PC1 out of three principal components which was accounted for 26.80% of total variance for 12 characters in 20 mutant and aromatic rice genotypes.

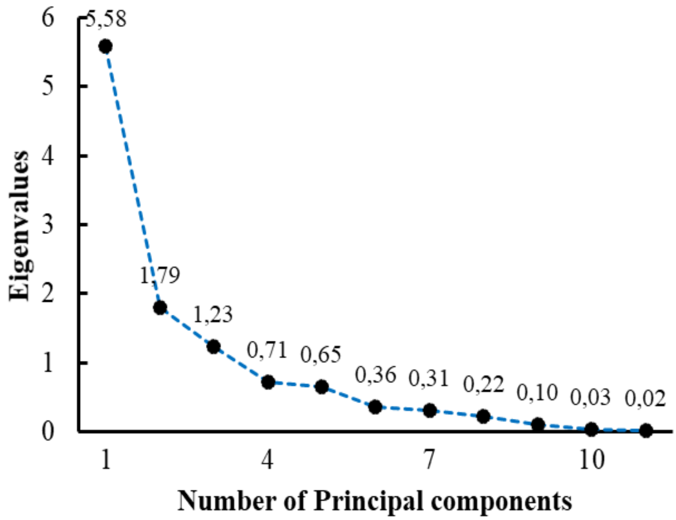


Figure 3. Eigenvalues of different principal components as shown by principal component analysis of rice landraces

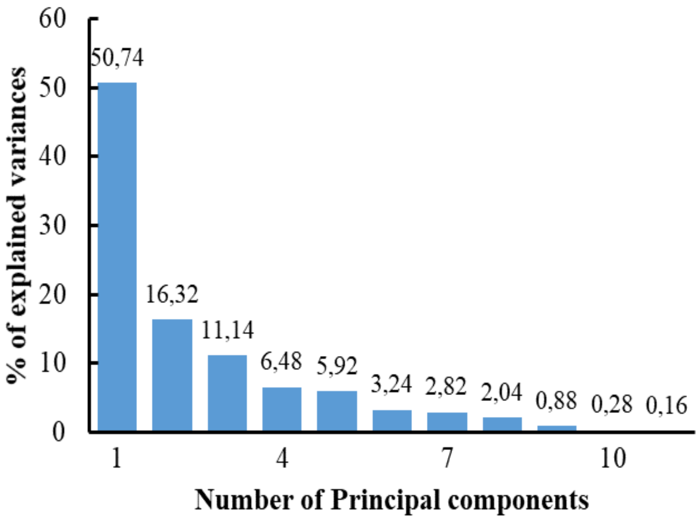
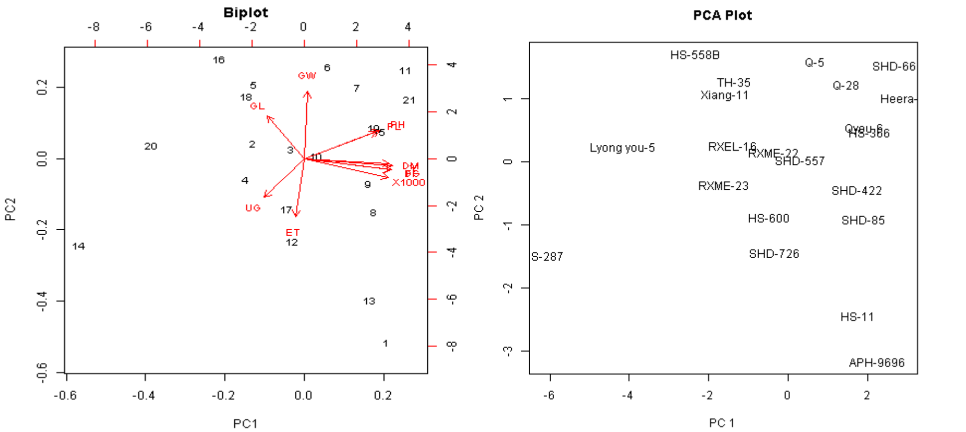


Figure 4. Contribution of each principal component to total explained variance in the phenotypic diversity of rice landraces

Table 3. Principal components for hybrid rice genotypes based on eleven quantitative characters

Statistics	PC1	PC2	PC3	PC4
Standard deviation	2.36	1.34	1.11	0.84
Proportion of Variance	0.51	0.16	0.11	0.06
Cumulative Proportion	0.51	0.67	0.78	0.85
Eigenvalues	5.58	1.79	1.23	0.71
Variables	Eigenvectors			
Plant Height (cm)	0.33	0.24	0.05	-0.19
Effective Tiller	-0.04	-0.50	-0.49	-0.49
Panicle length (cm)	0.32	0.23	0.20	0.24
Unfilled grain	-0.18	-0.34	0.55	-0.13
Filled grain	0.38	-0.10	0.06	0.17
Grain length (cm)	-0.16	0.37	-0.47	0.29
Grain Width (cm)	0.01	0.58	0.05	-0.70
Days to 50% flowering	0.38	-0.10	0.16	-0.06
Days to maturity	0.38	-0.05	0.11	-0.18
1000 grain weight (g)	0.37	-0.16	-0.25	-0.03
Yield (t/ha)	0.39	-0.06	-0.28	0.10



A) B)

Figure 5. Biplot (A) and Loading plot (B) of 11 quantitative characters in the principal component analysis of 21 rice genotypes. The eigenvectors were represented by red arrows showed how (the direction) and how much (the length) each traits contributes to the individual correlations represented by PC1 and PC2

Diversity analysis

A hierarchical clustering was performed to study the patterns of groupings of genotypes (Table 4). The dendrogram (Figure 6) was generated from Ward's linkage method based on squared Euclidean distance metric. By incision the dendrogram at 11 units distance, 21 rice genotypes were categorized into 3 main clusters, showing the availability of wide genetic diversity among the tested genotypes. Cluster I was the largest cluster which included fourteen genotypes. Meanwhile, Cluster II and Cluster III comprised five and two rice genotypes, respectively.

Table 4. Distribution of 21 hybrid rice genotypes in different cluster for various quantitative characters

Cluster	Genotypes#	Name of genotypes
I	14	APH-9696, RXME-22, Q-5, Q-28, SHD-85, SHD-422, SHD-557, SHD-661, SHD-726, HS-11, HS-366, HS-600, Qyou-6, Heera-2
II	5	RXEL-16, RXME-23, TH-35, HS-558B, Xiang-11
III	2	HS-287, Lyongyou-5

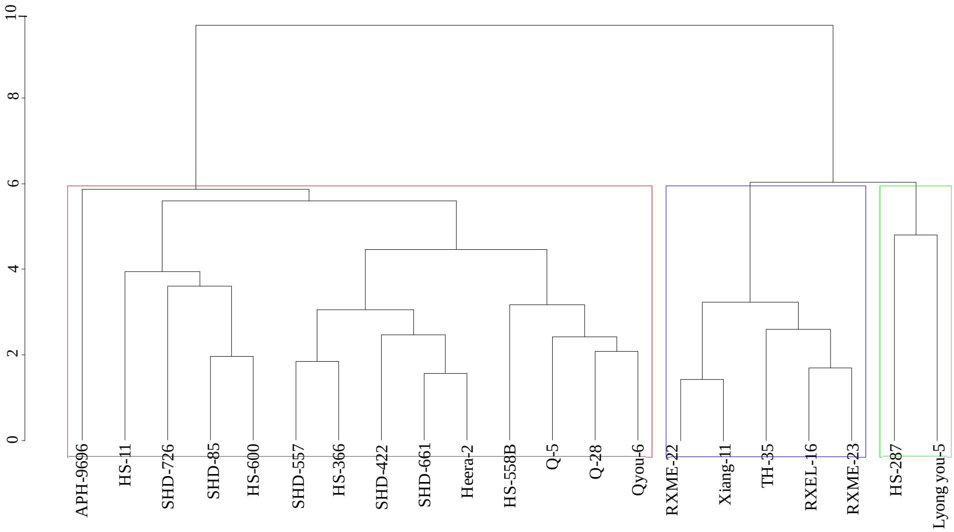


Figure 6. The hierarchical cluster analysis grouped the cultivars into 3 groups of 21 hybrid rice genotypes using Wards method and squared Euclidean distance (quantitative characters)

The cluster mean values showed a wide range of variations for all studied traits (Table 5). In more specific, cluster I containing genotypes producing the highest mean values for plant height, panicle length, filled grain and grain yield with moderate high rate of effective tillers. Except APH-9696, all the popular Chinese cultivars were included in cluster I. Meanwhile, genotypes belonging to India tend to fell together in the cluster II exhibited a high mean for the number of days to 50% flowering and maturity. clusters III contained Chinese cultivars HS-287 and Lyong you-5 either the highest or the lowest value for a particular character. However, considering the cluster means and Euclidean distances between the genotypes, cluster I exhibit high heterosis for yield potential. According to AbdEl-Aty *et al.* (2022) who found the variation in rice genotypes that showed significant and desirable particular combinability impacts, suggesting that it might be used in rice hybrid breeding program. Selecting the lines belonging to diverse clusters and showing high mean performance for desirable characters can be used in the selection of the parents for the breeding program (Dhakal *et al*., 2020; Hasan *et al.*, 2020; Shrestha *et al.*, 2021). So, based on this result, the genotypes under cluster I might have broad prospects for commercial application.

Table 5. The cluster means for yield and yield contributing traits in twenty-one hybrid rice genotypes

Traits	CLUSTER I	CLUSTER II	CLUSTER III	Contribution to diversity (%)
Plant Height (cm)	103.16	96.59	80.42	9.48
Effective Tiller	7.95	7.47	8	0.51
Panicle length (cm)	23.48	22.03	19.61	1.89
Unfilled grain	34.41	33.15	37.86	16.91
Filled grain	123.21	84.27	76	16.99
Grain length (cm)	8.73	9.21	8.93	0.54
Grain Width (cm)	2.57	2.59	2.6	0.20
Days to 50% flowering	114	120.79	109	3.40
Days to maturity	136	145.86	132.5	4.33
1000 grain weight (g)	25.22	17.8	12.42	2.23
Yield (t ha^{-1})	9.47	7.42	3.97	3.73

CONCLUSIONS

Determination of genetic diversity is important to select the more efficient plant cultivars for developing high yielding variety. In the present study, we quantified the magnitude of genetic diversity within the exotic hybrid rice genotypes those were collected from China and India by employing multivariate analysis based on elucidean cluster statistics and principal component analysis (PCA). Both analyses indicated the existence of the wide genetic diversity among the genotypes. PCA revealed that three of the eleven principal components having eigenvalue>1 contributed 78.19% of the variance. The analysis also identified plant height, panicle length, filled grain, grain width, thousand grain weight and yield as the most important traits for classifying the variation. Moreover, after assessing the yield attributing traits, the Chinese cultivars SHD-661, Qyou-6, Q-5 and Q-28 from cluster I had performed the better yield potentials among the studied rice cultivars. Therefore, the study will be highly beneficial to the rice breeders for selecting superior exotic hybrid rice genotypes to improve the rice grain productivity.

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FURTHER DISCOVERIES OF THE SUBTERRANEAN GENUS NIPHARGUS SCHIÖDTE, 1849) (FAM. NIPHARGIDAE) IN GREECE (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 331)

SUMMARY

The new member of the subterranean family Niphargidae (Crustacea: Amphipoda: Senticaudata) is discovered and described from the Maronia Cave, near Maronia town, Rhodopi district, NE Greece, *Niphargus beroni*, spec. nov., and its relation to some other members of genus *Niphargus* from adjacent regions of Balkan is discussed. By this way, the number of known members of the family Niphargidae in Greece is elevated to nearly 25 distinct taxa (genera, species and subspecies).

Keywords: Amphipoda**,** taxonomy, description, new species, *Niphargus beroni*, Greece, Balkan

INTRODUCTION

The fauna of the family Niphargidae (Crustacea: Amphipoda: Senticaudata) is relatively very rich in the subterranean waters of Greece (caves, springs, sulfuric springs, torrents, etc.). Thanks to study of this family by numerous scientists, speleologists and other investigators, the fauna of family Niphargidae in Greece reaching nearly 25 different taxa (genera, subgenera, species and subspecies) (G. Karaman, 2020). Exact number is not possible to establish, because various scientists used different criteria in recognition and delimitation of individual taxonomical categories, genera, subgenera, species and subspecies, or not accept some of these taxonomical categories. The similar observations appear also in using the terms “Group of taxa”, “*Niphargus* Complex”, “*Niphargus* aggregate”, etc., instead of the names genera, subgenera, tribes, etc.

The existing taxonomy was based on morphological characters to facilitate recognition and place of each taxon within certain taxonomical hierarchy. The discovery of every genetic molecular difference between various populations is

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not automatically evidence to establish a new taxon for each of them, what lead up to nominate numerous new taxa not or almost not differing to each other. That produce great problem in discovery and recognition of new taxa, because some newly established taxa are not clearly delimited to each other or to other similar taxa [delimitation can be negative or positive, but must exists], as the investigators can recognize which previously described species is similar, identic or not identic morphologically and/ or genetically to new taxon they intend to describe. The molecular/genetic taxonomy as well as morphological/ ecological taxonomy must recognize the level of differences necessary to recognize a different taxon, because in taxonomie it is possible to create numerous different new taxa based on the various limit of established differences. The use of combination of all methods is the best way in recognition of different taxa.

Recent attempt of fusing together some morphologically well defined genera of Niphargidae {*Pontoniphargus*, *Niphargus*, etc.) into one genus (Borko *et al*., 2019) based on only limited criteria is one example of these attempts to create a new taxonomy, where already some authors suggested that morphological description of new taxa is not necessary, etc. The establishing of all taxa above species is more or less conditional for recognition of distinct taxonomical hierarchy, and it can be made in numerous different ways (based on cytology, physiology, neurology, embryology, etc.). At the moment various genera, subgenera, species and subspecies are described in fauna of Niphargidae in Greece, but I will not analyze it here.

During our intensive research of Niphargidae family in Greece, we found one sample of genus *Niphargus* from cave near Maronia, collected in 1983 by P. Beron and V. Beshkov.

The speleologist and investigator Dr. Petar Beron, in his scientific work ”Faune cavernicole de la Grece” (2016: 12) mentioned: ”En Septembre 1983 j’ai visité (accompagné de V. Beshkov) les grottes à Kamari (Santorin) – Zoodochos I et II, la grotte de Maronia, la grotte d’Alistrati et un gouffre à Folegandros, avec de très bon résultat”, mentioning on page 22 the collecting of “?*Niphargus* sp.-nouv.: Grotte de Maronia (TW2).”

Evidently this famous Cave has been visited by various scientists many times and numerous new subterranean animals were collected and described from it. Beron explained (2016: 153): ”Maronia – le mont Ismaros (678 m) près du littoral de la mer Egée représente un promontoire méridional des Rhodopes. A présent la grotte de Maronia semble avoir une position isolée. Sa riche faune cavernicole est une indication que cette grotte est restée à sec pendant les fluctuations du littoral au Quaternaire.”

In this light, new species *Niphargus beroni* show some specific characters (mandible) but also some characters similar to some other *Niphargus* species from Serbia, Northern Macedonia and Bulgaria (uropods, etc.).

MATERIAL AND METHODS

The studied material was preserved by collectors in the 70% ethanol. The studied specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. Dissected body-parts were temporarily submersed in the mixture of glycerin and water for study. All illustrations were inked manually. After the study, these body-parts are submersed in Liquid of Faure on slides and covered by thin cover glass making definitive microscopic slides.

Some morphological terminology and setal formulae follow G. Karaman's terminology (Karaman, G., 1969) regarding the distal mandibular palpus article [A=A-setae on outer face; B=B-setae on inner face; D=lateral marginal D-setae; E=distal long E-setae], and later (2012b) regarding propodus of gnathopods 1 and 2 [S=corner S-spine; L=lateral slender serrate L-spines; M=facial corner M-setae; R=subcorner R-spine on inner face].

Terms "setae" and "spines" are used based on its shape, not origin. Our studies are based on the morphological, ecological and zoogeographical investigations.

In the REFERENCES we cited also the number of figures in each cited paper, because it is important to see in which of cited papers appear the figures important for determination.

TAXONOMICAL PART

AMPHIPODA SENTICAUDATA

FAMILY NIPHARGIDAE

NIPHARGUS BERONI, sp. nov.

Figures 1-7

MATERIAL EXAMINED: **Greece:**

Bu-7= Vil. Maronia, district Rhodopi, Maronia Cave, 1.10.1983, 5 exp. males, females (leg. P. Beron & V. Beshkov).

DIAGNOSIS OF SPECIES

Body slender, coxae shallow, coxa 4 unlobed, urosomal segments 1-2 with one dorsolateral seta. Epimoric plates 1-3 subrounded; pleopods 1-3 with elevated number of retinacula and peduncles scarcely setose. Maxilla 1: inner plate with one seta, outer plate with 7 spines (most of them with one lateral tooth, palpus reaching tip of outer plate-spines. Mandibular palpus with 3 distal setae on first article.

Gnathopods 1-2 small, with propodus not larger than corresponding coxa and dactylus with one median seta at outer margin. Dactylus of pereopods 3-7 short, with one spine or spine-like seta at inner margin. Article 2 of pereopods 5-7 longer than broad, ventroposterior lobe not distinctly developed.

Uropod 1 peduncle with dorsointernal row of setae; rami of uropod 1 in males paddle-shaped, in females normal. Uropod 3 in males long, with long distal article, in females distal article of outer ramus shorter. Telson in males deeply incised, lobes with row of distolateral slender spine-like setae and 1-2 facial mesial spine-like setae; in females lobe with 3-4 distal spines or spine-like setae, facial spines absent. Coxal gills recurved on gnathopod 2 and pereopod 4.

DESCRIPTION

MALE 7.2 mm (holotype): Body moderately slender, mesosomal segments naked, metasomal segments 1-3 with 3-4 dorsolateral short marginal setae (fig. 3E); urosomal segments 1-2 on each dorsolateral side with one seta, urosomal segment 3 naked (fig. 4F). Urosomal segment 1 at ventroposterior corner with one seta near basis of uropod 1 peduncle (fig. 4F).

Epimeral plates 1-3 subrounded, with several posterior marginal setae each (fig. 3E); epimeral plate 2 with 3 subventral spines, epimeral plate 3 with 5 subventral spines (fig. 3E).

Head with short rostrum and subrounded shallow lateral cephalic lobes, ventroanterior sinus developed (fig. 1A), eyes absent.

Antenna 1 not reaching half of body-length; peduncular articles 1-3 progressively shorter (ratio: 63:40:18), scarcely setose (fig. 1A); main flagellum consisting of 12+ articles (distal part of flagellum missing), some of articles with one short aesthetasc poorly visible. Accessory flagellum 2-articulated, short (fig. 1B).

Antenna 2 moderately slender, peduncular article 3 with distoventral seta; peduncular article 4 rather longer than article 5 (ratio: 48:40), both articles with several shorter setae each (fig. 1A). Flagellum slender, 7-articulated, longer than last peduncular article, bearing several short setae. Antennal gland cone short (fig. 1A).

Mouthparts well developed. Labrum broader than long, with poorly convex distal margin (fig. 4A). Labium with entire outer lobes, inner lobes shorter but well developed (fig. 4B).

Mandibles with triturative molar. Right mandible: incisor with 4 teeth, lacinia mobilis serrate, accompanied by 7-8 rakers (fig. 4D). Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth, accompanied by 8 rakers.Mandibular palpus well developed, 3-articulated: first article with 3 distal strong setae (fig. 4D); second article with 7 strong lateral setae; third article subfalciform, scarcely longer than second article, with nearly 10 lateral D-setae and 4 distal long E-setae, on outer (external) face by 2 A-setae, on inner (internal) face with 2 B-setae (fig. 4E).

Maxilla 1: inner plate with one seta, outer plate with 7 spines (6 spines with one lateral tooth, inner spine finely serrate; palpus 2-articulated: first article naked, second article reaching tip of outer plate spines, bearing 6 setae (fig. 1C).

Maxilla 2: both plates narrowed, bearing distal setae only (fig. 4C).

Maxilliped: inner plate short, with 2 distal spines mixed with several setae (fig. 5A); outer plate exceeding half of second palpus article, along mesial margin with nearly 12-13 marginal spines and several distal setae; palpus article 4 at inner margin with one long seta near basis of the nail (fig. 5A).

Coxae relatively short. Coxa 1 broader than long (ratio: 60:32), with subrounded ventroanterior corner bearing several short setae (fig. 2A). Coxa 2 broader than long (ratio: 66:42), with several marginal setae (fig. 2D).

Coxa 3 broader than long (ratio: 55:43), with 4-5 marginal short setae (fig. 1D). Coxa 4 much broader than long (ratio: 62:36), ventroposterior lobe absent (fig. 1E).

Coxae 5-7 short, not longer than coxae 1-4. Coxa 5 broader than long (ratio: 67:38), anterior lobe subrounded (fig. 3A). Coxa 6 smaller than coxa 5, bilobed, broader than long (ratio: 55:30) (fig. 3B). Coxa 7 entire, broader than long (ratio: 55:25) (fig. 3C).

Gnathopods 1-2 relatively small, smaller than corresponding coxae. Gnathopod 1: article 2 with long setae along both margins; article 3 with distoposterior bunch of setae (fig. 2A). Article 5 rather shorter than propodus (ratio: 60:71), at anterior margin with distal bunch of setae, at posterior margin with numerous setae (fig. 2B. Propodus nearly quadrate, longer than broad (ratio: 71:64), along posterior margin with 4 transverse rows of setae (fig. 2B). Palm slightly convex, inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 serrate L-spines and 3 corner facial M-setae (fig. 2C); on inner face by one short subcorner R-spine. Dactylus reaching posterior margin of propodus, with one median seta at outer margin and several short setae at inner margin (fig. 2B).

Gnathopod 2 poorly larger than gnathopod 1, article 2 with long setae at posterior margin and shorter setae along anterior margin; article 3 with distoposterior bunch of setae (fig. 2D). Article 5 triangular, rather shorter than propodus (ratio: 65:73), with 2 bunches of setae along anterior margin and numerous setae at posterior margin (fig. 2E). Propodus quadrate, poorly longer than broad (ratio: 73:68), along posterior margin with 6 transverse rows of setae (fig. 2E). Palm slightly convex, inclined rather less than half of propodus-length, defined on outer face by corner S-spine accompanied laterally by one serrate L-spine and 2 corner facial M-setae, on inner face by one short subcorner R-spine (fig. 2F). Dactylus reaching posterior margin of propodus, with one median seta at outer margin and several short setae at inner margin (fig. 2E).

Pereopods 3-4 moderately slender. Pereopod 3: article 2 with short anterior marginal setae and posterior longer setae (fig. 1D), articles 4-6 of different length (ratio: 52:31:38), bearing short setae and spines at both margins. Dactylus short and strong, much shorter than article 6 (ratio: 13:38), one slender spine at inner margin near basis of the nail and one short median seta at outer margin (fig. 1D.

Pereopod 4 rather shorter than 3, with hairiness like that of pereopod 3. Articles 4-6 of different length (ratio: 50:30:36); dactylus much shorter than article 6 (ratio: 16:36) (fig. 1E), inner margin with one slender spine near basis of the nail, outer margin with one median plumose seta; nail almost as long as pedestal (fig. 1F).

Pereopods 5-7 relatively short, progressively longer towards pereopod 7; pereopod 7 not reaching posterior end of the body.

Pereopod 5: article 2 longer than broad (ratio: 65:44), anterior margin slightly convex, posterior margin almost straight, bearing 7-8 marginal setae, ventroposterior lobe not distinctly developed (fig. 3A). Articles 4-6 of different length (ratio: 39:35:37), bearing scarce number of short spines and setae (fig. 3A); article 2 remarkably longer than article 6 (ratio: 65:37). Dactylus much shorter than article 6 (ratio: 13:37), with one spine-like seta at inner margin near basis of the nail, outer margin with one short median plumose seta (fig. 3A).

Pereopod 6 distinctly longer than 5, article 2 elongated, remarkably longer than broad (ratio: 80:44), with almost parallel lateral margins poorly convex, posterior margin with 8-9 short setae, anterior poorly convex margin with several stronger setae; ventroposterior lobe not distinctly developed (fig. 3B). Articles 4-6 of different length (ratio: 47:54:58), both margins with scarce number of short spines and setae. Article 2 distinctly longer than article 6 (ratio: 80:58). Dactylus much shorter than article 6 (ratio: 18:58), inner margin with one spine-like seta near basis of the nail, outer margin with one median seta (fig. 3B).

Pereopod 7: article 2 elongated, much longer than broad (ratio: 92:46), with almost parallel lateral margins, posterior margin with 11-12 short setae, anterior margin with 5-6 stronger setae; ventroposterior lobe not distinctly developed (fig. 3C). Articles 4-6 of different length (ratio: 50:60:73), both margins with scare number of short spines and setae. Article 2 remarkably longer than article 6 (ratio: 92:73). Dactylus much shorter than article 6 (ratio: 23:73), with one spine at inner margin near basis of the nail, outer margin with one median plumose seta (fig. 3D), nail shorter than pedestal.

Pleopods 1-3 with 3-4 retinacula each. Peduncles scarcely setose, peduncle of pleopod 1 with 3 anterior marginal setae (fig. 3F); peduncle of pleopod 2 naked (fig. 3G); peduncle of pleopod 3 with 1-2 setae at anterior margin (fig. 3H).

Uropod 1: peduncle with dorsointernal row of setae (fig. 4G). Rami dilated, paddle-shaped, nearly of equal length, with 4-5 distal short spines (fig. 4G).

Uropod 2 not dilated, rami with 4-5 distal short spines (fig. 4H).

Uropod 3 long, peduncle longer than broad, with some lateral and distal spine-like setae; inner ramus short, scale-like, with distal spine; outer ramus long, 2-articulated, linear, both articles with single short simple setae or spine-like setae; second article almost as long as first one (fig. 5B).

Telson deeply incised, nearly as long as broad; each lobe with one stronger spine and several long distolateral spine-like setae; on face of each lobe appear 1-2 spine-like setae (fig. 5C). A pair of short plumose setae is attached near the outer median margin of each lobe. Coxal gills on gnathopod 2 and pereopods 3-6 narrow, of different shape (fig. 1D, E, 3A, 3B).

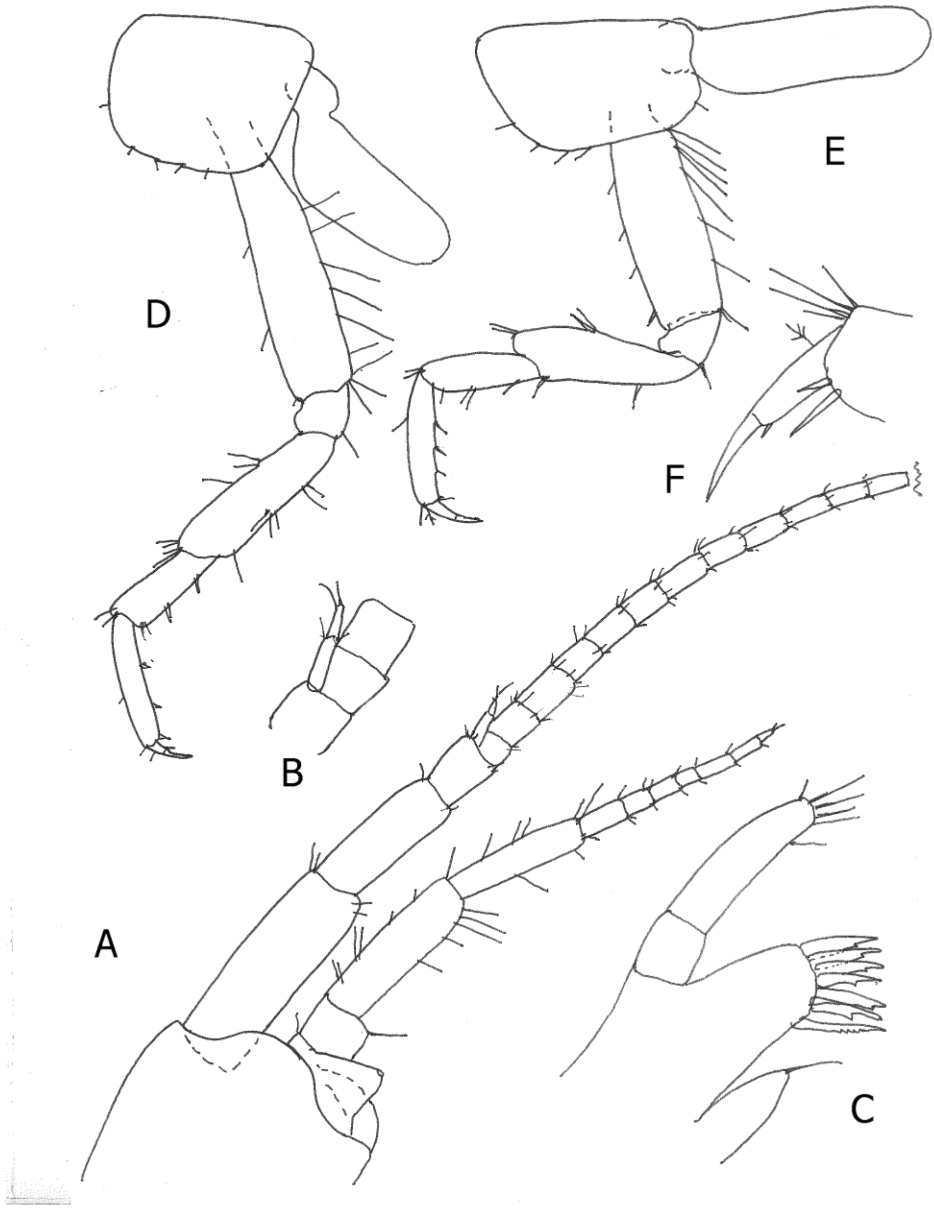

Fig. 1. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, male 7.2 mm (holotype): A= head with antennae 1-2; B= accessory flagellum; C= maxilla 1; D= pereopod 3; E-F= pereopod 4.

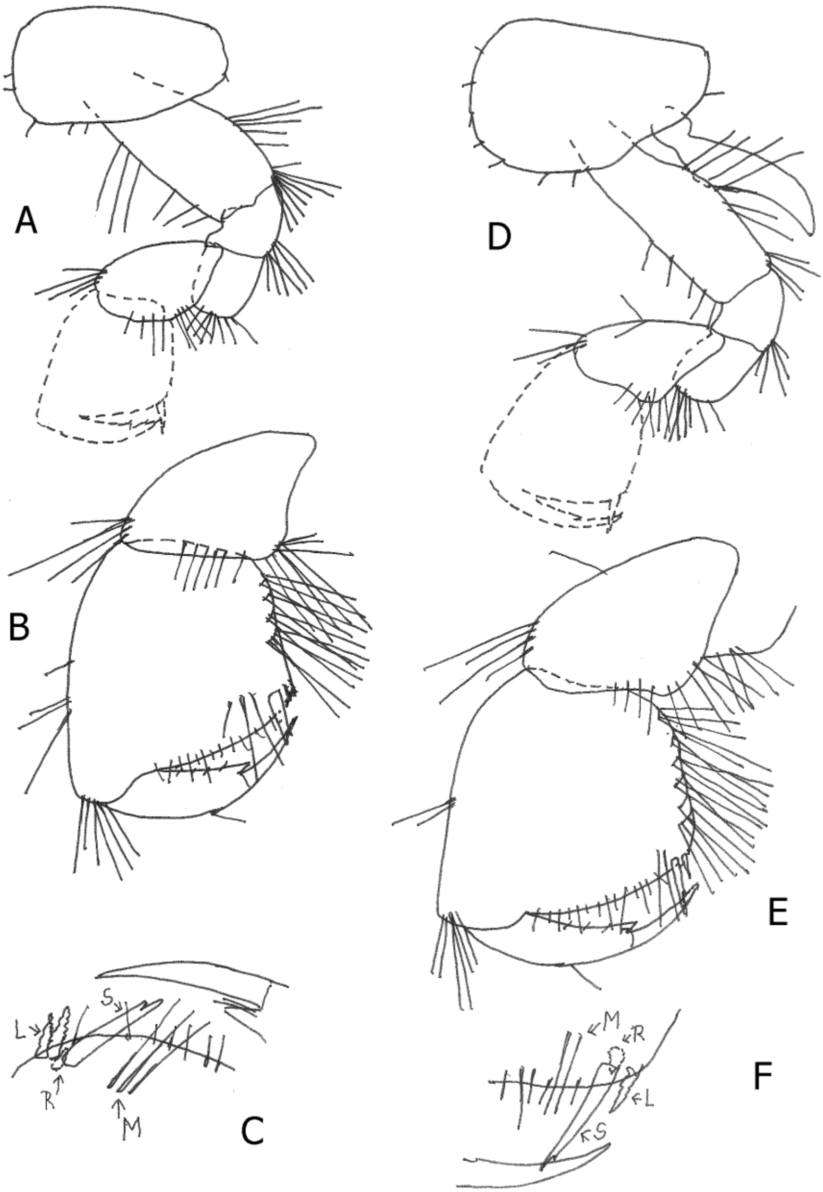

Fig. 2. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, male 7.2 mm (holotype): A-B= gnathopod 1; C= distal corner of gnathopod 1-propodus, outer face [S=- corner S-spine; L= lateral L-spines; M= corner facial M-setae; R= subcorner spine on inner face]; D-E= gnathopod 2; F= distal corner of gnathopod 2-propodus, outer face [S=- corner S-spine; L= lateral L-spines; M= corner facial M-setae; R= subcorner spine on inner face].

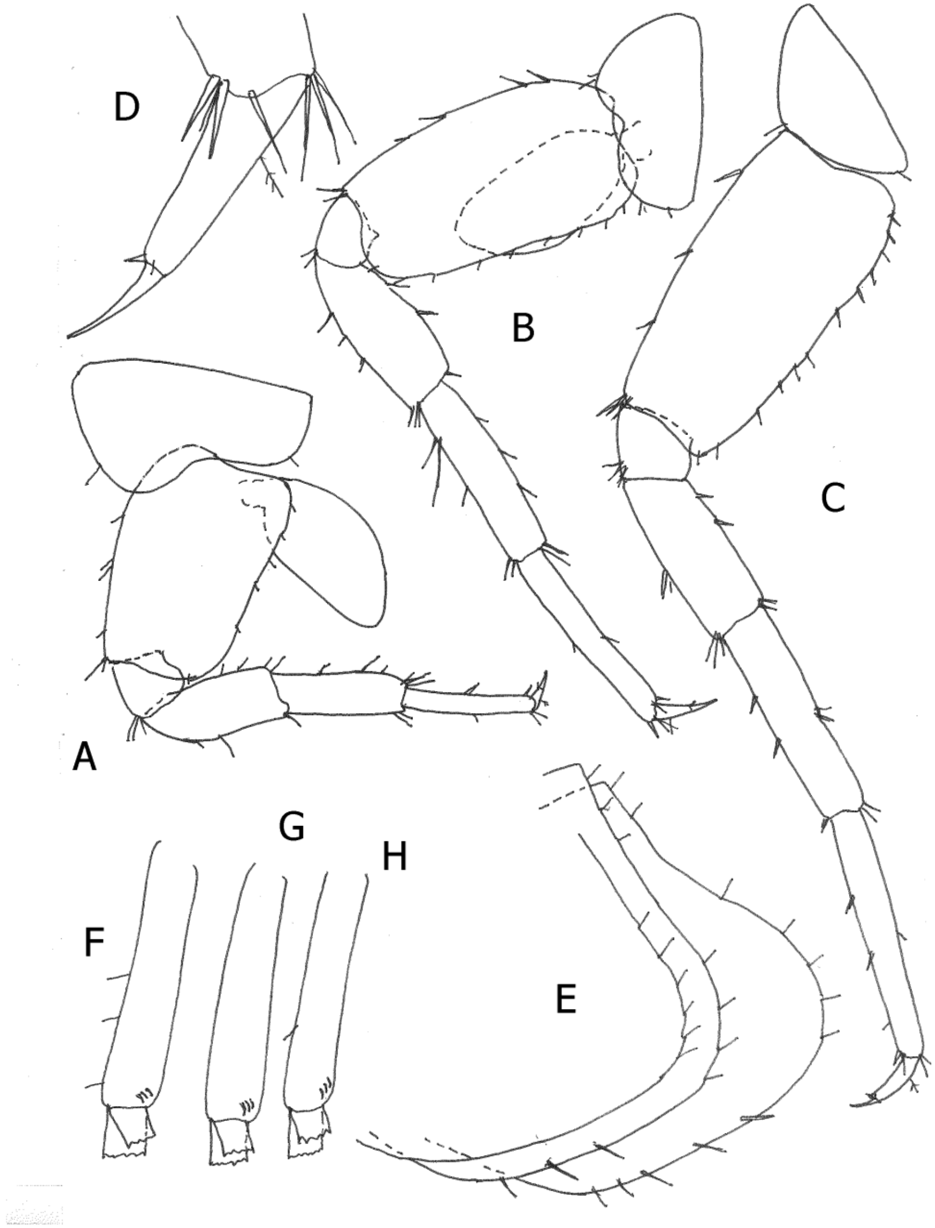

Fig. 3. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, male 7.2 mm (holotype): A= pereopod 5; B= pereopod 6; C-D= pereopod 7; E= epimeral plates 1-3; F-G-H= peduncle of pleopods 1-2-3.

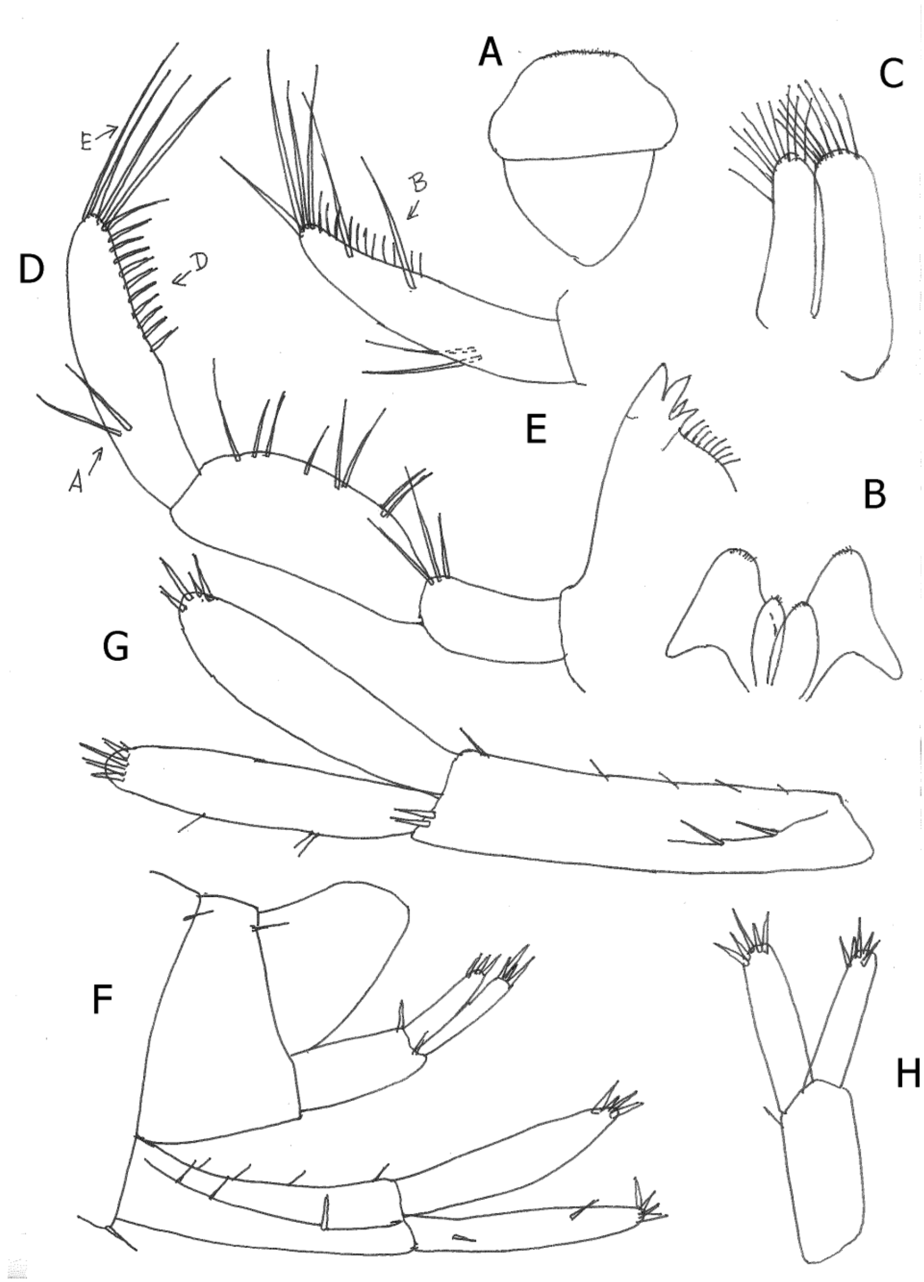

Fig. 4. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, male 7.2 mm (holotype): A= labrum; B=labium; C=maxilla 2; D=mandibular palpus, outer face [A=outer facial A-setae; B=inner facial B-setae; D=marginal D-setae; E=distal E-setae]; E=last palpus article, inner face [B=facial B-setae]; F=urosome with uropods 1-2; G= uropod 1; H= uropod 2.

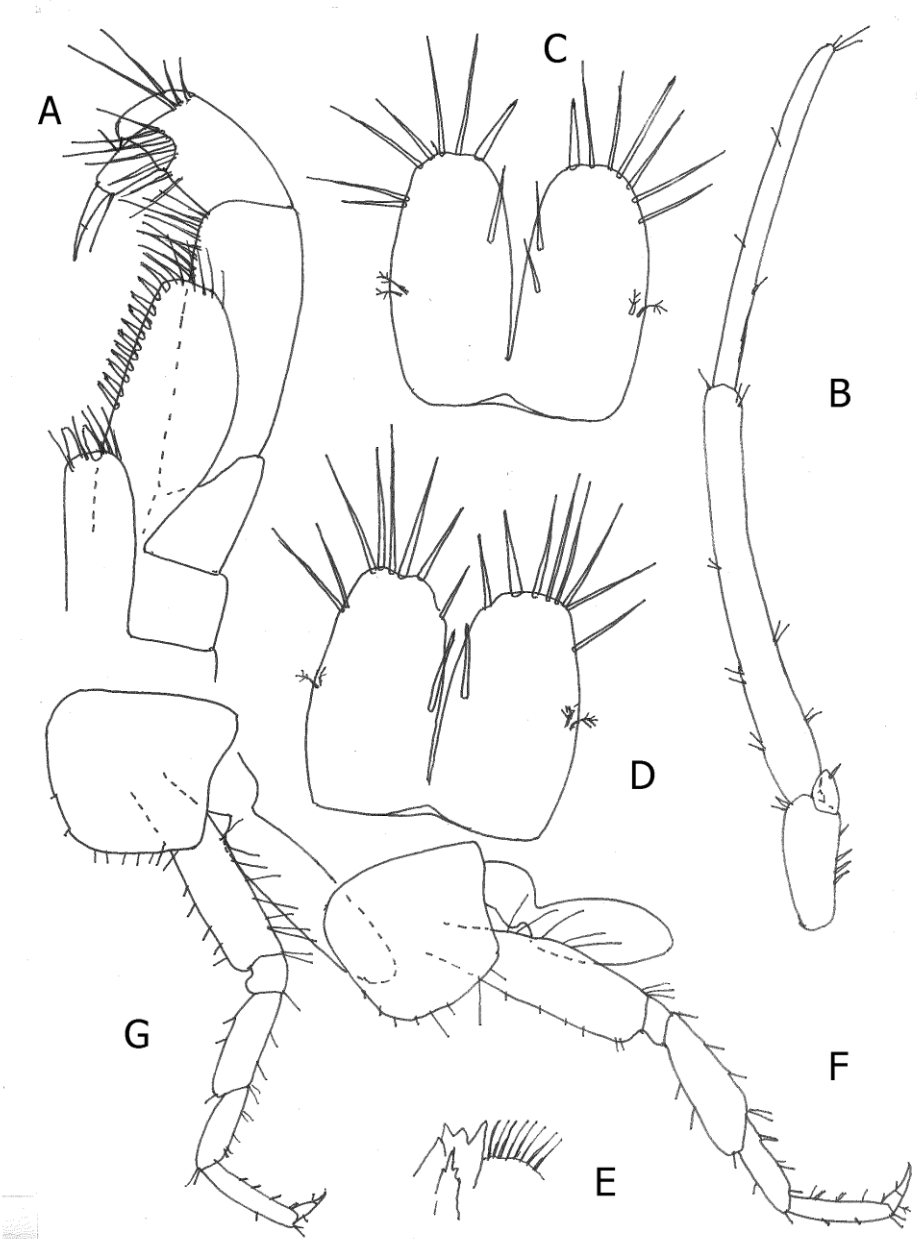

Fig. 5. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, male 7.2 mm (holotype): A= maxilliped; B= uropod 3; C= telson; D= telson, male 7.1 mm; E- tip of left mandible. **Female 5.4 mm (paratype):** F= pereopod 3; G= pereopod 4.

FEMALE 5.4 mm with setose oostegites (paratype): Head like that in male, metasomal segments with 2 dorsolateral posterior marginal setae each (fig. 7F); urosomal segments 1-2 with one dorsolateral seta on each side, urosomal segment 3 naked; Urosomal segment 1 with one ventroposterior seta near basis of uropod 1-peduncle.

Epimeral plates 1-3 subrounded, like these in male; epimeral plate 2 with 2 subventral spines, epimeral plate 3 with 3 subventral spines (fig. 7F).

Antenna 1 like that in male, almost reaching half of body-length, peduncular articles 1-3 progressively shorter; main flagellum consisting of 15-16 articles scarcely setose (some of them with one short, poorly visible aesthetasc); accessory flagellum 2-articulated, short. Antenna 2 like that in male, scarcely setose, peduncular article 4 poorly longer than article 5; flagellum longer than last peduncular article, consisting of 8 articles; antennal gland cone short.

Mouthparts like these in male (fig. 5E). Mandibular palpus article 1 with 3 distal setae (fig. 7A); article 2 rather shorter than article 3, with 10 lateral setae; article 3 subfalciform, with nearly 12 lateral D-setae and 4-5 distal E-setae, on outer face with 2 A-setae, on inner face with 2 B-setae (fig. 7A).

Labrum, labium, maxillae 1-2 and maxilliped like these in male. Maxilla 1 inner plate with one seta, outer plate with 7 spines (5-6 spines with one lateral tooth, 1 spine with 1-2 lateral teeth, inner spine serrate; palpus 2-articulated, reaching tip of outer plate-spines, with 6 distal setae.

Coxae relatively short. Coxa 1 broader than long (ratio: 50:29), with subrounded ventroanterior corner (fig. 7B). Coxa 2 rather broader than long (ratio: 48:41), with several short marginal setae (fig. 7D). Coxa 3 rather broader than long (ratio: 50:43), with 7-8 marginal setae (fig. 5F). Coxa 4 broader than long (ratio: 53:45), with 8-9 marginal short setae, ventroposterior lobe absent (fig. 5G). Coxa 5 broader than long (ratio: 70:48), bilobed (fig. 6A). Coxa 6 smaller than 5, bilobed, broader than long (ratio: 60:40) (fig. 6B). Coxa 7 entire, broader than long (ratio: 55:31) (fig. 6C).

Gnathopods 1-2 relatively small, with propodus smaller than corresponding coxa. Gnathopod 1 poorly smaller than gnathopod 2; article 2 with long setae at both margins (fig. 7B), article 3 with distoposterior bunch of setae. Article 5 almost as long as propodus, anterior margin with 2 groups of setae (fig. 7C), and numerous setae at posterior margin. Propodus poorly trapezoid, longer than broad (ratio: 71:63), along posterior margin with 3 transverse rows of setae (fig. 7C). Palm convex, inclined half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 L-spines and corner facial 2 M-setae (fig. 7C), on inner face by one short subcorner R-spine. Dactylus reaching posterior margin of propodus, with one median seta at outer margin and several short setae at inner margin (fig. 7C). Gnathopod 2: article 2 with row of shorter setae at anterior margin and row of long setae at posterior margin, article 3 with distoposterior bunch of setae (fig. 7D). Article 5 as long as propodus, with 2 groups of setae at anterior margin and numerous setae at posterior margin (fig. 7E). Propodus rather trapezoid, nearly as long as broad, with 5 transverse rows of setae at posterior margin (fig. 7E). Palm convex, inclined nearly half of propodus-length, defined on outer margin by corner S-spine accompanied laterally by 2 slender L-spines and 3 corner facial M-setae, on inner face by one subcorner R-spine (fig. 7E). Dactylus reaching posterior margin of propodus, with one median seta on outer margin and several short setae at inner margin (fig. 7E).

Pereopods 3-4 moderately short. Pereopod 3 with long setae at posterior margin; article 3 with distoposterior bunch of setae. Articles 4-6 of different length (ratio: 40:25:31), scarcely setose (fig. 5F). Dactylus much shorter than article 6 (ratio: 14:30), at inner margin with one slender spine-like seta near basis of the nail, on outer margin with one median plumose seta (fig. 5F).

Pereopod 4 poorly shorter than pereopod 3, article 2 anterior margin with shorter setae, posterior margin with longer setae; article 3 with distoposterior bunch of setae (fig. 5G). Articles 4-6 of different length (ratio: 32:24:30), articles scarcely setose, setae short. Dactylus much shorter than article 6 (ratio: 13:30), with one spine-like seta at inner margin near basis of the nail, outer margin with one median seta (fig. 5G).

Pereopods 5-7 progressively longer towards pereopod 7. Pereopod 5: article 2 slightly longer than broad (ratio: 63:45), along posterior margin with 8-9 setae, anterior margin with 6-7 spine-like setae, ventroposterior lobe not developed (fig. 6A). Articles 4-6 of different length (ratio: 40:35:38), both margins with short spines and setae. Article 2 longer than article 6 (ratio: 63:38). Dactylus shorter than article 6 (ratio: 23:38), at inner margin with one spine-like seta near basis of the nail, outer margin with one median plumose seta, nail shorter than pedestal (fig. 6A).

Pereopod 6: article 2 distinctly longer than broad (ratio: 79:51), posterior poorly convex margin with 9-10 short setae, anterior margin with 6 setae, ventroposterior lobe not distinctly developed (fig. 6B). Articles 4-6 of different length (ratio: 49:55:65), with short spines and single short setae along both margins. Article 2 longer than article 6 (ratio: 79:65). Dactylus shorter than article 6 (ratio: 25:65), at inner margin with one spine-like seta near basis of the nail, outer margin with one median plumose seta (fig. 6B).

Pereopod 7: article 2 longer than broad (ratio: 90:55), posterior poorly convex margin with nearly 16 short setae, anterior margin with 4-7 setae, ventroposterior lobe not distinctly developed (fig. 6C). Articles 4-6 of different length (ratio: 48:63:79), along both margins with single short spines and setae. Article 2 longer than article 6 (ratio: 90:79). Dactylus shorter than article 6 (ratio: 32:79), at inner margin with one slender spine near basis of the nail, outer margin with one median plumose seta (fig. 6C), nail shorter than pedestal. Pleopods 1-3 with 4 retinacula each. Peduncle of pleopods 1-3 scarcely setose, like these in male.

Uropod 1: peduncle with distal spines. Rami narrowed, normal, of subequal length, not paddle-shaped, both with 4-5 short distal spines (fig. 6D).
Uropod 2: rami narrowed, nearly of subequal length, with 3-4 distal spines (fig. 6E).

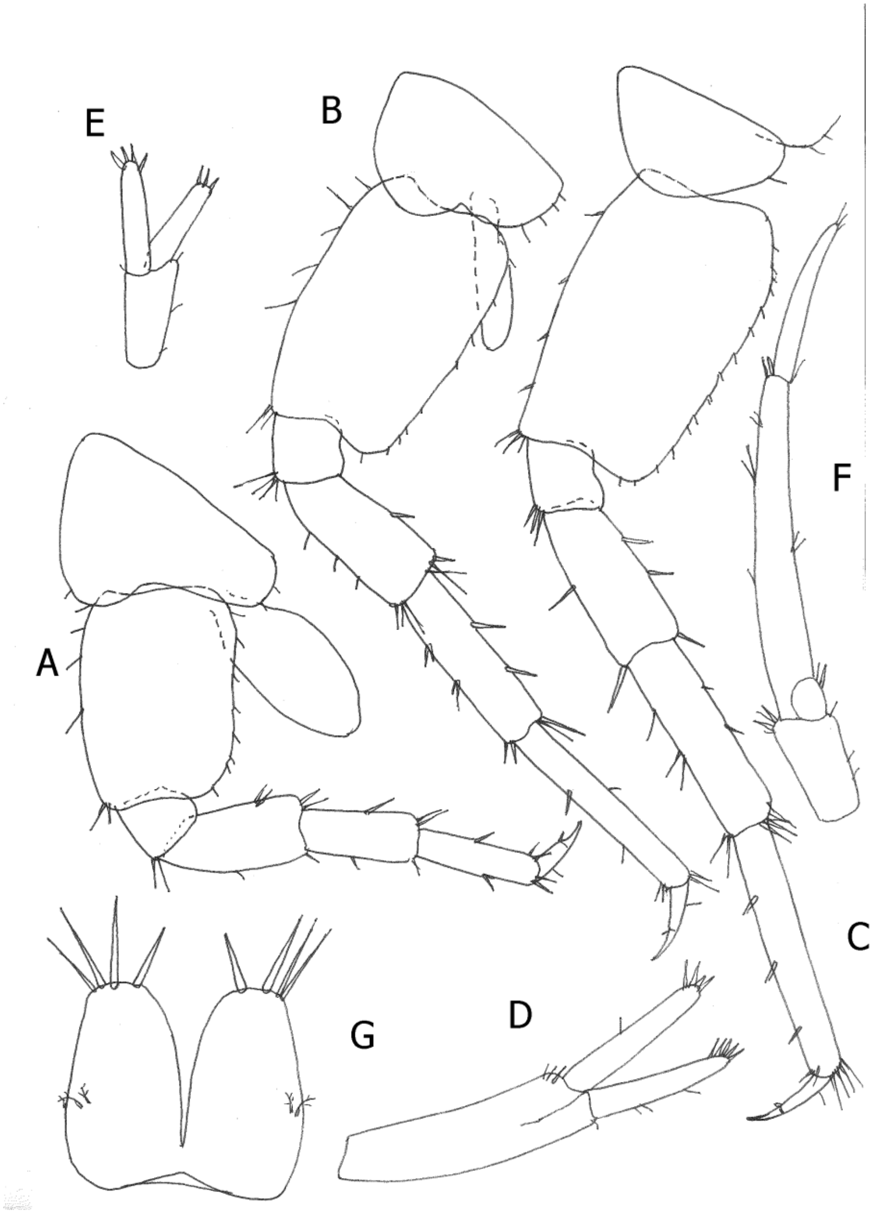

Fig. 6. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, female 5.4 mm (paratype): A= pereopod 5; B= pereopod 6; C= pereopod 7; D= uropod 1; E=uropod 2; F= uropod 3; G= telson.

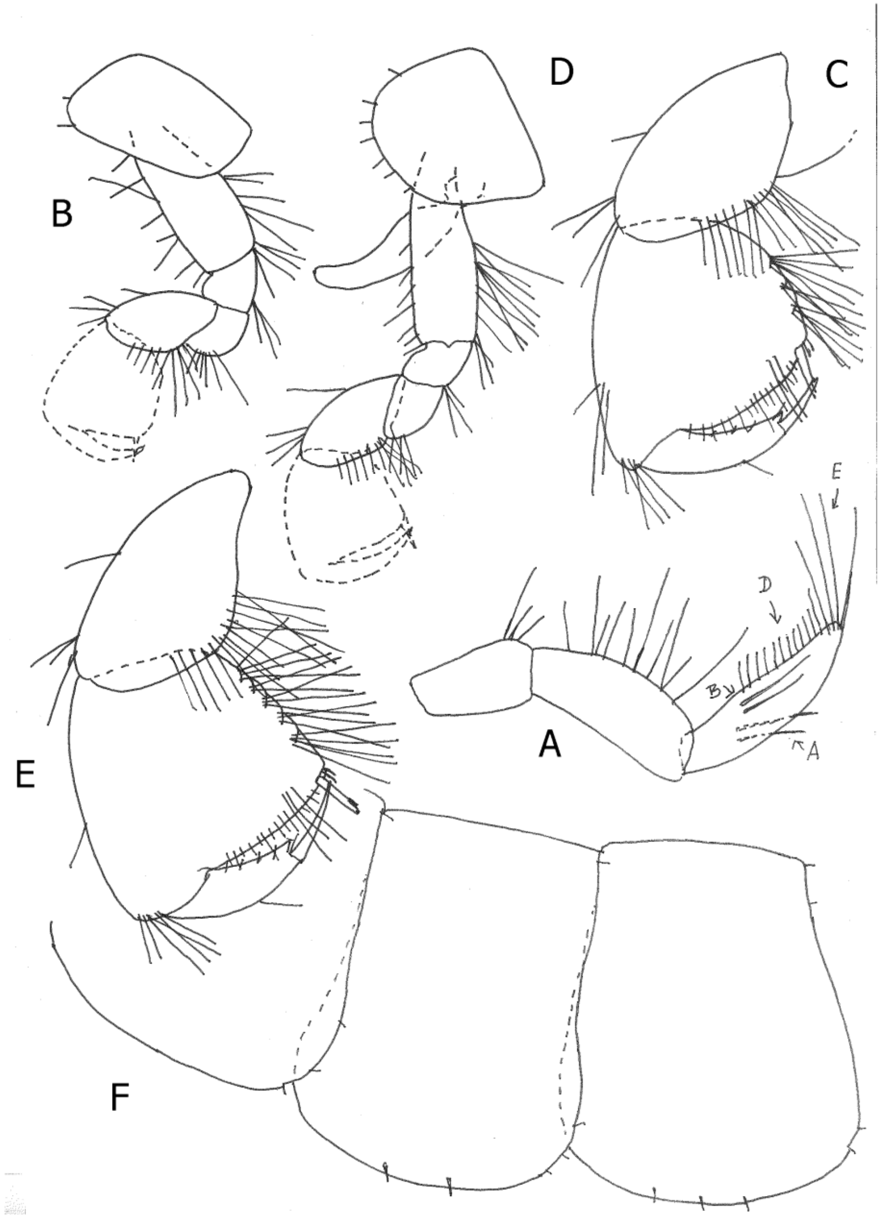

Fig. 7. *Niphargus beroni*, sp. nov., Maronia Cave, Maronia, Greece, female 5.4 mm (paratype): A=mandibular palp, inner face [B=inner facial B-setae; A=outer facial A-setae]; B-C= gnathopod 1; D-E= gnathopod 2; F=epimeral plates 1-3.

Uropod 3 slender, but shorter than that in male. Peduncle longer than broad, with several short distal spines; inner ramus short, scale-like, with distal spine and seta (fig. 6F); outer ramus 2-articulated: first article long, along both margins with single short simple setae or single small spine; second article nearly half as long as first one, with 3 distal short simple setae.

Telson relatively short, deeply incised, broader than long (ratio: 70:63), each lobe with 4 distal spine-like setae of various size; lateral and facial spines or setae absent; a pair of short plumose setae is attached near the median outer margin of each lobe (fig. 6G).

Coxal gills like these in male (figs. 5F, G.; 6A, B; 7D). Oostegites broad, with long marginal setae.

VARIABILITY. Main flagellum of antenna 1 in male and female not reaching half of body-length, consisting of 14-15 articles. Epimeral plates 2 and 3 with 2-5 subventral spines. Gnathopod 2 propodus with 1-2 L- spines. Maxilliped: inner plate with 2-3 distal spines, palpus article 4 with 1-2 unequal setae near basis of the nail.

Lobes of telson in males with 2-3 facial setae in mesial part of each lobe (fig. 5C, D); lobes of telson in female with 4 distal spine-like setae only, facial spines or setae absent.

HOLOTYPE (male 7.2 mm) and PARATYPE (female 5.4 mm with setose oostegites) are deposited in Karaman`s Collection in Podgorica (Crna Gora, Montenegro), under the No. BU-7.

REMARKS AND AFINITIES.

Schellenberg (1933) described a new subspecies *Niphargus aquilex tauri*, sp. nov. from cave in Taurus Mts., Turkey, Asia Minor.

Karaman, G. (1973) in his redescription of lectotype of *Niphargus tauri* Schell. [Cave in Taurus Mts., Asia Minor, Turkey] figured and mentioned: ”Die Àste der I. Uropoden sind schmal und sehr schwach dorsoventral abgeplattet, aber nicht schaufelformig verbreitert, auch bei sehr alten Exemplaren”.

Karaman, S. (1943) described specimens from Kragujevac (Serbia) as *Niphargus aquilex tauri* Schellenberg, 1933. Observing later that these specimens are not identic with specimens of *N. aquilex tauri* Schell., he nominated it (S. Karaman, 1950: 90) as a new subspecies *N. tauri kragujevensis*, ssp. nov. [loc. typ.: Kragujevac, Serbia].

In the same publication S. Karaman described 2 other similar different taxa: *Niphargus tauri jurinaci*, ssp. nov. [loc. typ.: Crni Lug, Croatia] and *Niphargus tauri medvednicae*, ssp. nov. [loc. typ.: Zagrebačka gora Mts. near Zagreb, Croatia]. Both these taxa are with normal developed rami of uropod 1 in males.

S. Karaman described (1959) a new subspecies *Niphargus tauri osogovensis,* ssp. nov. [loc. typ.: Osogovo Mts., eastern part of North Macedonia] with normal developed rami of uropod 1 in males.

Karaman, S. & Karaman, G. (1959) described a new subspecies *Niphargus tauri pecarensis* [loc. typ.: Pečara dupka Cave, Bulgaria], discovered later in Serbia also (G. Karaman, 1999).

Karaman, G. (1992) redescribed *N. kragujevensis kragujevensis* S. Kar. 1950 from type-locality, and described new subspecies *N. kragujevensis remus*, ssp. nov. [loc. typ.: Prekonoga, Svrljig, eastern Serbia]; both of them with dilated rami of uropod 1 in males.

Karaman, G. (1998) redescribed *Niphargus pecarensis* S. & G. Karaman, 1959 and described a new subspecies *N. p. occultus,* ssp. nov. [loc. typ.: Matinee, spring, Montenegro, Crna Gora]. Later he discovered *N. pecarensis* in Serbia (G. Karaman, 1999) and Romania (G. Karaman, 2022).

Kenderov, Lj. & Andreev, S. (2015) described a new species *Niphargus cvetkovi* sp. nov. [loc. typ.: source "Cheshma Gorgoritsa" near the village Novi Han, east of Sofia, Bulgaria], with dilated rami of uropod 1 in males.

But all these mentioned species are without setae on first palpus article of mandible, despite the fact that some of these species are with normal or more or less inflated paddle shaped rami of uropod 1 in males, or are with densely or scarce hairiness of peduncle in pleopods 1-3.

The new species *Niphargus beroni,* in spite of similarity to the species mentioned above, differs from all of them by presence of setae on first article of mandibular palpus. Otherwise, this character is present in only a few known *Niphargus* species: *Niphargus timavi* S. Karaman, 1954 [loc. typ.: subterranean waters of Timavo River on Italian/ Slovenian border] (G. Karaman, 1985), *Niphargus religiosus* G. Karaman, 2012a [loc. typ.: Uragavaz Gecidi, Ballidag (Kastamonu), Turkey], etc., but all these species differ remarkably from *N. beroni* by various morphological characters.

DERIVATIO NOMINIS. The new species *Niphargus beroni* is dedicated to Prof. Dr. Petar Beron from Sofia (Bulgaria), scientist, speleologist and investigators of the subterranean fauna of Balkan and other parts of the World, who collected sample of this new species together with prof. Beshkov.

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INSPECTION OF BURNING AREAS CAUSED BY FOREST FIRE IN DOI SUTHEP-PUI NATIONAL PARK THROUGH DATA OBTAINED FROM LANDSAT 8 SATELLITE AND NORMALIZED BURN RATIO

SUMMARY

The objective of this research is to inspect areas burned by a forest fire in Doi Suthep-Pui National Park in Muang District, Chiang Mai Province, Thailand, with data from the Landsat 8 satellite and Normalized Burn Ratio (NBR). The research was conducted by analyzing data from the Landsat 8 satellite between 2017 and 2021 in pre- and postfire periods. Data were analyzed using NBR before analyzing to find burning areas through ΔNBR. The accuracy of results was evaluated point by point using false color composites (7(R),5(G),4(B)) by creating 60 random points. Analysis results revealed that accuracy in the study period was 81.66%, 81.66%, 83.33%, 81.66%, and 86.66%, respectively. Moreover, when inspecting the consistency of analysis results using the Kappa statistic, it was found that, during the study period, the Kappa coefficient was 0.78, 0.81, 0.82, 0.82, and 0.84, respectively. As a result, it could be concluded that false color composites (7(R), 5(G), 4(B)), NBR, and Δ NBR can reliably and consistently be used for specifying areas burned by forest fires. Relevant agencies can apply this case study to inspect further burning areas caused by forest fires.

Keywords: Remote Sensing, Doi Suthep-Pui National Park, Forest Fire, Normalized Burn Ratio, Landsat 8

INTRODUCTION

Forest fire refers to a fire that spreads throughout the natural forest or forestry plantations independently without control. A forest fire cannot be caused if the following three factors, Fire Triangle (Linta *et al.,* 2021), are lacking. There are two fundamental causes of forest fires including (1) natural causes, such as lightning, friction between branches, volcanic eruption, clash of stones, the

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incidence of sunlight to quartz, sunlight shining through water droplets, a chemical reaction in a swamp forest, spontaneous combustion of living creatures) and (2) anthropogenic causes, for example, forest product collection, ignition, and negligence (Ruthamnong, 2019). The rapid population growth increases the need to utilize forest resources; therefore, forests are reclaimed as agricultural and residential areas. The expansion of communities destroys forests rapidly (Department of National Parks, Wildlife and Plant Conservation, n.d.). The remaining forest areas are degenerated and transformed into forests with lower humidity, for example, mixed forests, deciduous forests, and forests with large amounts of grass or meadow that can fuel forest fires (Untamedscience, n.d.).

Furthermore, poverty in rural areas forces people to increasingly rely on forests for living by collecting forest products, hunting wild animals, or clearing forests for farming. These activities require fire, so they become the causes of forest fires. Consequently, the frequency and severity of forest fires are capable natural mechanisms that adjust themselves to balance the forest ecosystem (Urbancreature, 2021; 3armyarea-rta, n.d.). For this reason, forest fires cause severe damage to forests and the environment. Currently, the frequency of forest fires in Thailand has sharply increased, becoming a factor disturbing ecosystem balance. Significant impacts of forest fire are: 1) impact on soil, forest, water, and wild animals as well as small creatures in forests; 2) impact on assets, health, human life, economy, society, and tourism; and 3) impact on global climate (Baankluayonline, 2020). Subsequent impacts cause severe drought in the rainy season, off-season rain, and flooding. As a result, forest fire has become an internationally significant problem that is currently realized by all sectors (Flannigan *et al.*, 2000).

Doi Suthep-Pui National Park has forest and mountain areas. Around 70% of the total area consists of granite mountains and limestone mountains covered by various kinds of forest, including hill evergreen forest, dry evergreen forest, mixed forest, deciduous forest, and deciduous forest mixed with coniferous forest. Forested areas consist of natural forests, forestry plantations, and naturally restored forests (Chiang Mai Provincial Office, 2021). Each year, the number of forests is decreased by 0.02% (Kongmeesup, 2020) due to several causes, mainly deforestation and forest fire. Forest fires impact southern Thailand every year, especially during the dry season from the beginning of November to the end of April (Panyakam & Pongsawat, 2022). Forest fire mainly occurs in mixed forest, deciduous forest, and forestry plantations (Royal Forest Department, n.d.), so it is necessary to build firebreaks in these areas and encourage cooperation with local people regarding forest burning to reduce the severity of forest fire and smoke caused by forest burning (Gnews, n.d.). In addition to data from land surveys, remote sensing technology can be used to evaluate burning forest areas (Boer *et al.*, 2009; Mohammadi *et al.*, 2014; Tariq *et al.*, 2021a and Tariq *et al.*, 2021b).

Currently, remote sensing technology, such as satellite data, is rapidly and efficiently developing (Debbarma and Debnath, 2021; Potić *et al.*, 2021 and Thangaraj and Karthikeyan, 2021). Remote sensing technology contains basic physics principles on an electromagnetic wave used as media to obtain data without direct contact with objects (ESA, 2016; Elachi and Zyl, 2021; University of Lucknow, n.d.). Moreover, remote sensing technology can record data from vast areas at a lower cost than land surveying (Laosuwan *et al*., 2016; Rotjanakusol and Laosuwan, 2018a; Rotjanakusol and Laosuwan, 2018b; Rotjanakusol and Laosuwan, 2019; Prohmdirek *et al*., 2020; Uttaruk and Laosuwan, 2020; Jomsrekrayom *et al*., 2021, Sangpradid *et al*., 2021; Suriya *et al.,* 2021; Uttaruk *et al.,* 2022).

MATERIAL AND METHODS

Research area

Doi Suthep-Pui National Park (Figure 1) is located in Muang Chiang Mai District, Mae Rim District, and Hang Dong District, with a total area of 261 km^2 (Thai National Parks, 2023) and consisting of a plentiful forest. Although it is located near Chiang Mai's downtown, most forest areas are on complex mountain ranges, such as Doi Suthep and Doi Pui. The general climate of Doi Suthep-Pui National Park is influenced by southwest monsoon-blowing humidity and clouds causing rain and northeast monsoon blown from China causing coldness and drought.

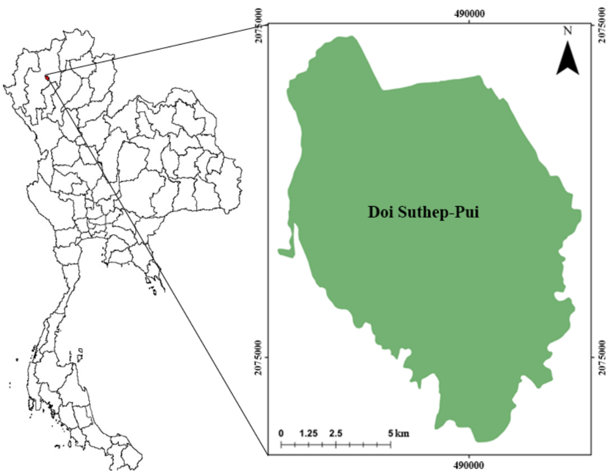


Figure 1. Research area

Satellite Data

The Landsat 8 satellite was launched on May 30th, 2013, under the management of the United State Geological Survey (USGS). It orbits 705 km above the earth's surface with two types of recording devices, including an Operational Land Imager (OLI) (30 m) and a Thermal Infrared Sensor (TIRS) (100 m), and repeats coverage every 16 days. It consists of 11 bands, and the first to seventh and ninth bands consist of wavelengths ranging from 0.43–0.45 µm

(Coastal Aerosol), 0.45–0.51 µm (Blue), 0.53–0.59 µm (Green), 0.64–0.67 µm (Red), 0.85–0.88 µm (Near Infrared: NIR), 1.57–1.65 µm (SWIR 1), 2.11–2.29 µm (SWIR 2), and 1.36–1.38 µm (Cirrus) with a spatial resolution of 30 meters. The eighth band has a wavelength from 0.50–0.68 µm (Panchromatic) with a spatial resolution of 15 m. The tenth and eleventh bands have wavelengths from 10.60–11.19 µm (Thermal Infrared 1, TIRS 1) and 11.50–12.51 µm (TIRS 2) with a spatial resolution of 100 m (USGS, 2022). This research was conducted by analyzing data obtained from the Landsat 8 satellite (https://earthexplorer.usgs.gov/) from December 2017 to 2021 in the pre-fire and from March 2017 to 2021 postfire periods.

Conversion of Digital Number as Reflectance

Conversion of Digital Number as Reflectance is considered the process of data preparation before conducting analysis (pre-processing) by converting Digital Number (DN) obtained from Landsat 8 to Reflectance by using Equation 1 (Ruthamnong, 2019). Subsequently, obtained data were calculated to find adjustments with sun elevation, as shown in Equation 2 (Ruthamnong, 2019).

$$\rho\lambda' = M\rho.Qcal + A\rho \quad (1)$$

Where:

$\rho\lambda'$= TOA planetary reflectance, without correction for solar angle

$M\rho$=Band-specific multiplicative rescaling factor from the metadata (REFLECTANCE_MULT_BAND_x, where x is the band number)

$A\rho$=Band-specific additive rescaling factor from the metadata (REFLECTANCE_ADD_BAND_x, where x is the band number)

Qcal=Quantized and calibrated standard product pixel values (DN)

$$\rho\lambda = (\rho\lambda'/\cos(\theta_{SZ})) = (\rho\lambda'/\sin(\theta_{SE})) \quad (2)$$

Where:

$\rho\lambda$=TOA planetary reflectance

θ_{SE}=Local sun elevation angle. The scene center sun elevation angle in degrees is provided in the metadata (SUN_ELEVATION).

θ_{SZ}=Local solar zenith angle; $\theta_{SZ} = 90° - \theta_{SE}$

Normalized Burn Ratio (NBR) Analysis

NBR is a kind of index designed to find burning areas and evaluate fire severity. This method is similar to the Normalized Difference Vegetation Index (NDVI), but NBR use of the wavelength of Near Infrared (NIR) and Short Wave Infrared (SWIR), as shown in Equation 3 (Keeley, 2009). This method will give plants high Reflectance in Near Infrared (NIR) and low Reflectance in Short Wave Infrared (SWIR). On the other hand, the burning area will have low Reflectance in Near Infrared (NIR) and high Reflectance in Short Wave Infrared

(SWIR). Generally, high NBR indicates a small number of plants, empty spaces, and burning areas. This research analyzed data obtained from Landsat 8 satellite in pre-fire and postfire periods using Normalized Burn Ratio (NBR). Subsequently, burning areas were analyzed using ΔNBR, as shown in Equation 4 (Smith *et al*., 2007).

$$NBR=(NIR-SWIR)/(NIR+SWIR) \quad (3)$$

Where:
NIR=Near-infrared (NIR) reflectance
SWIR=Shortwave-infrared (SWIR) reflectance

$$\Delta NBR=NBR\ pre\text{-}fire - NBR\ postfire \quad (4)$$

Where:
ΔNBR=NBR difference value
NBR pre-fire=NBR value before the fire
NBR postfire=NBR value after fire

Validity assessment

False color composite

A false-color composite was created to inspect the validity of burning areas caused by forest fires. Data obtained from Landsat 8 satellite were used for creating a false-color composite by mixing red, green, and blue wavelengths with SWIR NIR and Red (false color composite: 7(R),5(G),4(B)) wavelength. This kind of false color composite can show burning areas clearly with purple to dark purple in the manner of spreading from the center of a forest fire or the direction of active spreading shown with orange to red. Forest areas were shown with green, deciduous forest areas with light purple, pink, and white, and open areas with white, pink, or light orange. Water sources were shown with dark blue, and agricultural areas were shown with white, light green, or dark green based on types of appearance of plants and the density of cover crops (Uttaruk *et al*., 2022).

Random forest

Random forest is a machine-learning model developed from decision tree. Random forest adds the number of trees giving more efficiency and accuracy (Belgiu and Dragut, 2016; Deur *et al*., 2020). Currently, random forest is highly preferred (Phiri *et al*., 2018). The principle of random forest is building a model from various sub-models of decision trees. While performing prediction, each decision tree will perform predictions, and the prediction results can be calculated by voting output that is mainly selected by decision tree in case of classification or finding the mean from each decision tree's output in the case of regression (Pal, 2005). In this research, the sample size was calculated before sampling representatives of burning areas in the research area by using random points through random forest in the ArcGIS program. Subsequently, data from sample groups were classified into two types: points of burning areas and non-burning areas for evaluating accuracy.

Consistency Test with Kappa

The Kappa coefficient, or Cohen's Kappa coefficient as it is officially known (Banko, 1998), is the statistic used for testing the consistency level in two data groups. In some cases, it may be used for comparing the evaluation of the same dataset from two evaluators. In comparison, the Kappa coefficient is not required to be based on the hypothesis that interested data were distributed using normal distribution or non-parametric statistics. Results obtained from the Kappa coefficient explained the consistency of the two datasets.

RESULTS AND DISCUSSION

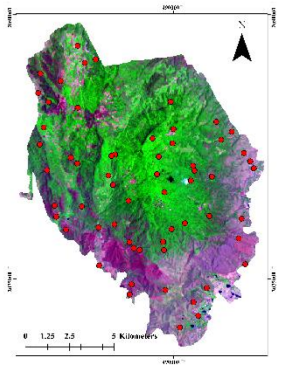
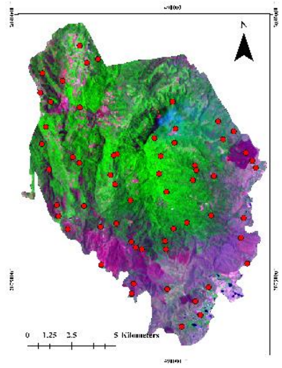
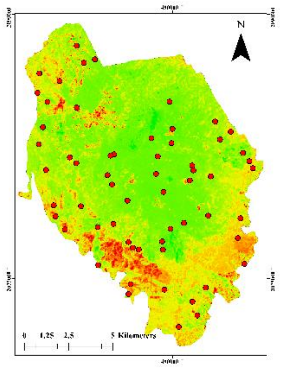
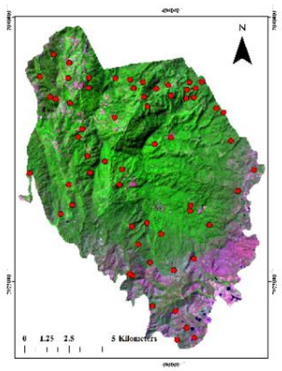
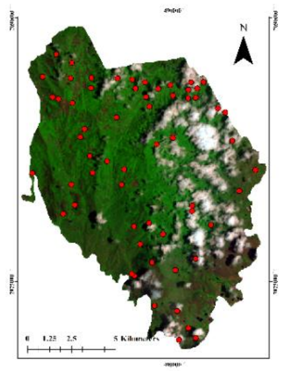
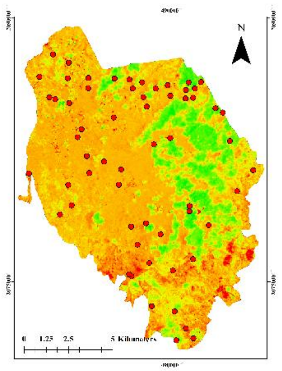
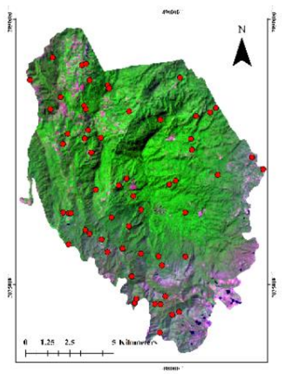
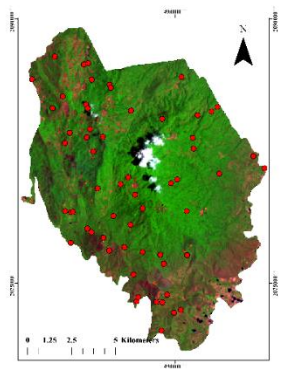
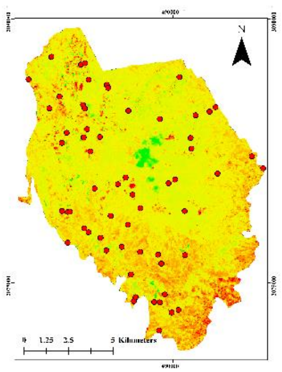
Results of False Color Composite for Inspecting Burning Areas

Results of the analysis on burning areas caused by forest fires between 2017 and 2021 through data obtained from Landsat 8 satellite were used for creating false color composite by mixing colors in red, green, and blue wavelength with SWIR NIR and Red (false color composite: 7(R),5(G),4(B)) wavelength in pre-fire and postfire periods based on NBR and ΔNBR. Moreover, the accuracy of burning areas caused by forest fires was tested by using random points with 60 points. Analysis results are shown in Figure 2.

From Figure 2, it was found that false color composite 7(R),5(G),4(B) created from data obtained from Landsat 8 satellite for inspecting burning areas caused by forest fire was shown in purple to dark purple in the manner of spreading from the center of a forest fire or the direction of active fire's spreading that were shown with orange to red. Forest areas were shown with green. Deciduous forest areas were shown with light purple, pink, and white. Open areas were shown with white, pink, or light orange. Water sources were shown with dark blue, and agricultural areas were shown with white, light green, or dark green based on the types and appearance of plants and the density of cover crops.

Moreover, when analyzing the results of false color composite 7(R), 5(G), and 4(B) from Landsat 8 satellite data with ΔNBR through 60 random points, it was found that there were 49 points of 60 points of burning areas that were results of analysis on ΔNBR in 2017 calculated to be 81.66%.

Results of the analysis on ΔNBR in 2018 revealed that 49 points of 60 points of burning areas were calculated to be 81.66%. Results of the analysis on ΔNBR in 2019 revealed that there were 50 points out of 60 points of burning areas calculated to be 83.33%. Results of the analysis on ΔNBR in 2020 revealed that 49 points of 60 points of burning areas were calculated to be 81.66%. Results of the analysis on ΔNBR in 2021 revealed that there were 52 points out of 60 points of burning areas calculated to be 86.66%.

NBR pre-fire in 2017	NBR postfire in 2017	ΔNBR in 2017
		
NBR pre-fire in 2018	NBR postfire in 2018	ΔNBR in 2018
		
NBR pre-fire in 2019	NBR postfire in 2019	ΔNBR in 2019
		
NBR pre-fire in 2020	NBR postfire in 2020	ΔNBR in 2020

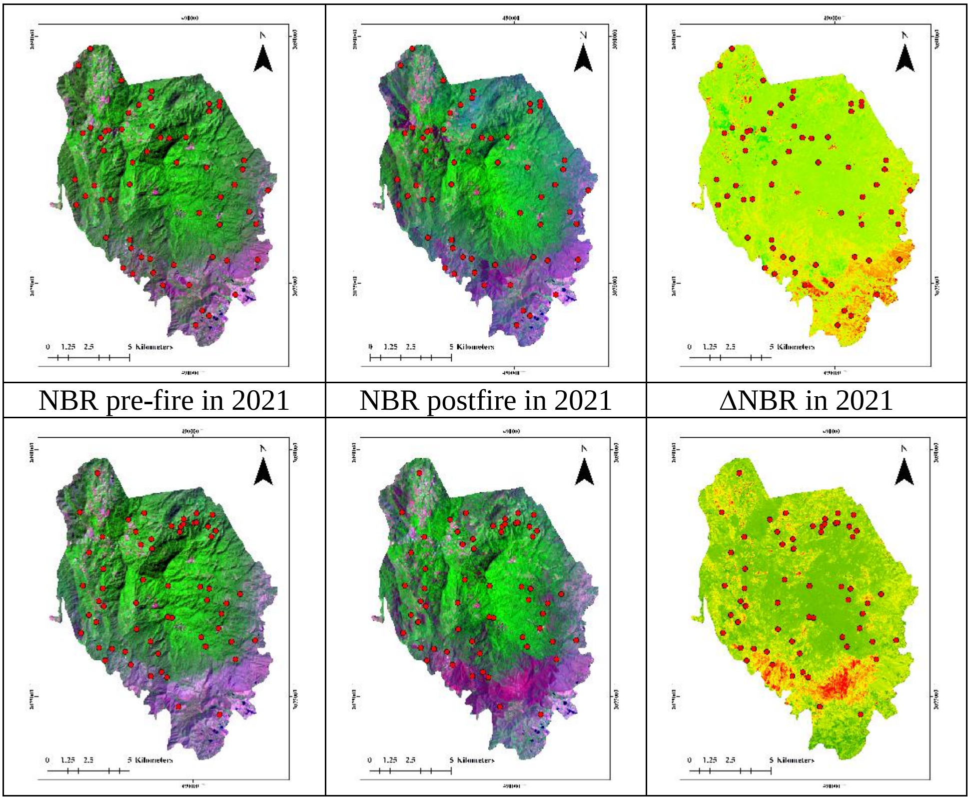


Figure 2. NBR pre-fire, NBR postfire, and ΔNBR between 2017 and 2021

Results of False Color Composite for Inspecting Burning Areas

In this research, results of analysis on data obtained from Landsat 8 satellite (false color composite) and ΔNBR between 2017 and 2021 were tested on consistency using Kappa. From a total of 60 random points, they were divided into 20 points of burning areas, 20 non-burning areas, and 20 forest areas. Random points were determined by creating random points in the ArcGIS program (Data Management Tools > Feature Class > Create Random Points) (ArcGIS Desktop, 2021) and comparing them with point-by-point by visual analysis. Results of the consistency test with Kappa are shown in Table 1.

From ΔNBR 2017 data, it was found that overall accuracy was 81.66%. It was also found that the Kappa coefficient was 0.78. When considering the class of the burning areas, it was found that the producer's accuracy was 73.91%, with an omission error of 26.09%, a user's accuracy of 85%, and commission error of 15%.

From ΔNBR 2018 data, it was found that overall accuracy was 81.66%. It was also found that the Kappa coefficient was 0.81. When considering the class of burning areas, it was found that the producer's accuracy was 78.94 %, with an omission error of 21.06%, a user's accuracy of 75%, and commission error of 25%.

Table 1. Consistency test with Kappa

ΔNBR in 2017					
ΔNBR	Forest area	Non-forest area	Burning area	Sum	User's accuracy
Forest area	**17**	1	2	20	85%
Non-forest area	1	**15**	4	20	75%
Burning area	1	2	**17**	20	85%
Sum	19	18	23	**60**	
Producer's accuracy	89.47%	83.33%	73.91%		
Kappa statistics	0.78				
ΔNBR in 2018					
ΔNBR	Forest area	Non-forest area	Burning area	Sum	User's accuracy
Forest area	**18**	1	1	20	90%
Non-forest area	1	**16**	3	20	80%
Burning area	3	2	**15**	20	75%
Sum	22	19	19	**60**	
Producer's accuracy	81.81%	84.21%	78.94%		
Kappa statistics	0.81				
ΔNBR in 2019					
ΔNBR	Forest area	Non-forest area	Burning area	Sum	User's accuracy
Forest area	**17**	2	1	20	85%
Non-forest area	2	**18**	0	20	90
Burning area	1	4	**15**	20	75%
Sum	20	24	16	**60**	
Producer's accuracy	85%	75	93.75%		
Kappa statistics	0.82				
ΔNBR in 2020					
ΔNBR	Forest area	Non-forest area	Burning area	Sum	User's accuracy
Forest area	**16**	1	3	20	80%
Non-forest area	5	**14**	1	20	70%
Burning area	0	1	**19**	20	95%
Sum	21	16	23	**60**	
Producer's accuracy	76.19%	87.5%	82.60%		
Kappa statistics	0.84				
ΔNBR in 2021					
ΔNBR	Forest area	Non-forest area	Burning area	Sum	User's accuracy
Forest area	**18**	0	2	20	90%
Non-forest area	1	**16**	3	20	80%
Burning area	0	2	**18**	20	90%
Sum	19	18	23	**60**	
Producer's accuracy	94.73%	88.88%	78.26%		
Kappa statistics	0.82				

From ΔNBR 2019 data, it was found that overall accuracy was 83.33%, and it was also found that the Kappa coefficient was 0.82. When considering the class of burning areas, it was found that the producer's accuracy was 93.75%, with an omission error of 6.25%, a user's accuracy of 75%, and a commission error of 25%.

From ΔNBR 2020 data, it was found that overall accuracy was 81.66%. It was also found that the Kappa coefficient was 0.84. When considering the class of burning areas, it was found that the producer's accuracy was 82.60%, with an omission error of 17.40%, a user's accuracy of 95%, and commission error of 5%.

From ΔNBR 2021 data 2022, it was found that overall accuracy was 86.66%. It was also found that the Kappa coefficient was 0.82. When considering the class of burning areas, it was found that the producer's accuracy was 78.26%, with an omission error of 21.74%, a user's accuracy of 90%, and commission error of 5%.

The burning index that uses 2 data periods before and after the burning area is analyzed, such as the ΔNBR index, provides the results of the analysis of the burning area that is more accurate than using data at a single period to calculate. Schepers *et al.* (2014) and Liu *et al*. (2020) concluded that the burning index was used from satellite images recorded during the visible and near-infrared waves. Intense than the combustion index that uses the near-infrared wave with middle infrared. In addition, the accuracy of the analysis of the combustion area depends on the selection of the index that uses the wave range to analyze the appropriate combustion space, with the time of the analyzed data must be appropriate as well.

However, if the rehabilitation of the burned area, there should be a distance between the data that is analyzed and compared with the NBR and the ΔNBR index and, therefore, should be more than one year apart because it will be able to monitor the rehabilitation, including the violence of combustion. In addition, this study is in line with the research on “A New Metric for Quantifying Burn Severity: The Relativized Burn Ratio” (Parks *et al*., 2014), the study “Evaluation of Spectral Indices for Assessing Fire Severity in Australian Temperate Forests” (Tran *et al.,* 2018), and The study “Evaluating the Differenced Normalized Burn Ratio for Assessing Fire Severity Using Sentinel-2 Imagery in Northeast Siberian Larch Forests” (Delcourt *et al.*, 2021), concluded that NBR and ΔNBR can identify burning areas caused by forest fire effectively.

CONCLUSIONS

Usually, in the event of any combustion caused by any cause, poor management or control may lead to spreading. After burning, the soil's color will be changed to grey or even black due to heat on the soil caused by the burning of leaves or scraps from harvesting crops that covers the soil. After burning, these fuels will leave black soot and ashes. If satellite data are used for inspecting areas after the scene, burned areas will be able to be visually observed and separated more clearly. However, visual interpretation may be insufficient to survey and inspect for assess damages because each wildfire can cause massive damage. As a result, researchers and related persons often use data from aerial photography or satellite to inspect or assess initial damages. This research aims to apply Normalized Burn Ratio (NBR), and Difference Normalized Burn Ratio (ΔNBR) because many researchers have applied them to analyze burned areas via satellite data. Normalized Burn Ratio, or NBR, was designed to focus on burned areas, and its calculation was similar to that of the Normalized Difference Vegetation Index (NDVI). However, NBR uses Nir Infrared (NIR) and Short Wave Infrared (SWIR) because plentiful plants will have a high reflection in NIR wavelength, but its reflection will be low in SWIR wavelength. Therefore, we can separate burned areas from other areas correctly.

This research was conducted to inspect burning areas caused by forest fires in the national park through data obtained from Landsat 8 satellite and Normalized Burn Ratio in the area of Doi Suthep-Pui National Park in Muang Chiang Mai District, Mae Rin District, and Hang Dong District in Chiang Mai province during 2018–2021. The study concluded that data from Landsat 8 satellite and Normalized Burn Ratio could specify burning areas that occurred in the research area and false color composite 7(R), 5(G), 4(B), NBR, and ΔNBR from data obtained from Landsat 8 satellite could represent burning areas. Moreover, results of the consistency test with Kappa between 2018 and 2021 revealed high accuracy, with an overall accuracy of 81.66%, 81.66%, 83.33%, 81.66%, and 86.66%, respectively. In addition, it was also found that the Kappa coefficient was 0.78, 0.81, 0.82, 0.82, and 0.84, respectively. Anyone interested and related agencies could efficiently apply this method to inspect further burning areas caused by forest fires.

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CLONAL VARIATIONS IN GROWTH CHARACTERISTICS OF ANATOLIAN BLACK PINE SEED ORCHARDS

SUMMARY

Seed orchard is an important cultivated seed source to produce genetically seed crop for forestry practices. Growth characteristics have important roles in management of seed orchards and seed harvesting. Growth variations among seed orchards, and among clones within orchard were investigated in three seed orchards of Anatolian black pine [*Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe] based on tree height, diameter at base, diameter at breast height, and crown diameter measured at end of growth period of 2022. The seed orchards were established consist of 30, 30 and 34 clones selected phenotypically from natural stands in 1994 (SO1), 1991 (SO2) and 1985 (SO3), respectively.

Averages of tree height (9.30 m), diameter at base (31.91 cm), diameter at breast height (27.13 cm), and crown diameter (7.09 m) were the highest in SO3, while they were the lowest as 5.78 m, 24.50 cm, 20.26 cm, 5.00 m, respectively in SO2. However, clones and ramet within clone in seed orchards showed large differences for the characteristics. For instance, clonal averages of crown diameter ranged from 3.55 m to 6.46 m in SO2. SO1 had the highest annual increments for the characteristics except of tree height of SO3. Results of analysis of variance indicated significant ($p \leq 0.05$) differences among the orchards, and among clones within orchard except of tree heights of SO1 and SO2 for the characteristics. Tree height was more homogeneous than the other characteristics according to the Duncan's multiple range test.

Positive and significant ($p \leq 0.05$) relations were found between the pairs of tree height, diameter at base, diameter at breast height, and crown diameter in each seed orchard both phenotypic and genotypic. Results of the study could be used in establishment and management of seed orchards.

Keywords: Breeding, Correlation, Diameter, Height, Ramet, Variance

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INTRODUCTION

According to the latest forestry inventory, Turkey has 23.1 million ha forest area of which 9.6 million ha is unproductive. Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe] is an important tree species in Turkish forestry and forestry practices by 4.2 million ha natural distribution including which of 31.9% is unproductive (OGM, 2022). The species is also one of the main species of “National Tree Breeding and Seed Production Programme” (Koski and Antola, 1993). It has 73 selected seed stands at 9375.7 ha, and 57 established seed orchards at 475.8 ha (ORTOHUM, 2022). The seed sources play important roles to produce improved seeds and conversion of unproductive forest to productive forest in the species. Seed orchard is a plantation of assumed superior genotypes established to produce tree seeds. Typically, it is clones or seedlings from selected trees, isolated to reduce pollination from outside sources, even ground and wide spacing to facilitate cone harvest, and managed for early, easily accessible, and abundant seed production (Kang and Bilir, 2021). Seed orchard can be also defined shortly as one of the cultivated seed sources. Orchard managers and owners focus on growth and reproductive characteristics in the cultivation practices such as spacing, pruning, tree density. For instance, significant relations are reported between growth and reproductive characteristics in different forest tree species (e.g., Bhumibhamon, 1978; Nikkanen and Velling, 1987; Dutkuner *et al*., 2008; Bilir *et al*., 2008 and 2017; Kang and Bilir, 2021). Growth characteristics balanced by different forestry practices are important factors in management practices, and in harvesting of cheap seed crop. Estimation of variations of growth characteristics is one of the main stages for decision on present and future management practices in seed orchards.

Variations among clones within seed orchard, and among orchards were estimated for tree height, diameter at base, diameter at breast height, and crown diameter, together with correlations among the characteristics in three seed orchards of Anatolian black pine to contribute seed orchard management practices of the species in this study.

MATERIAL AND METHODS

The study was carried out in three Anatolian black pine clonal seed orchards established at 8m x 8 m spacing by 30, 30 and 34 clones selected phenotypically from natural seed stands in 1994, 1991 and 1985, respectively (Table 1, Figures 1 and 2).

Table 1. Some details of the orhards

Orchard	Latitude (N)	Longitude (E)	Number of clones	Number of seedlings	Area (ha)	Establishment year
SO1	37°57′58″	30°34′25″	30	2800	17.6	1994
SO2	38°04′19″	30°05′46″	30	2000	13.8	1991
SO3	37°09′28″	29°40′19″	34	1248	9.4	1985

Figure 1. Studied seed orchard, SO3

Figure 2. Location of the seed orchards

Five ramets of each clone were sampled in the orchards. Tree height (**TH**), diameter at base (**D_0**), diameter at breast height (**$d_{1.30}$**), and crown diameter (**CD**) of the sampled ramets were measured at end of growth period of 2022.

Collected data was performed by following model of analysis of variance (ANOVA) to compare seed orchards, and clones within orchard for the growth characteristics at SAS (SAS, 2004).

$$Y_{ijk} = \mu + F_i + B(F)_{j(i)} + e_{ijk}$$

Where Y_{ijk} is the observation from the k^{th} ramet of the j^{th} clone in the i^{th} orchard, μ is overall mean, $B(F)_{j(i)}$ is effect of the j^{th} clone in the i^{th} orchard, and e_{ijk} is random error.

Seed orchards and clones within orchard were grouped by Duncan's multiple range test (Duncan, 1955) for the characteristics based on results of analysis of variance.

Phenotypic correlations (r_p) between the pairs of the tree height, diameter at base, diameter at breast height and crown diameter were estimated in each orchard as (Falconer, 1989):

$$r_p = \frac{COV_{f(x,y)}}{\sqrt{\sigma^2_{f(x)}}\sqrt{\sigma^2_{f(y)}}}$$

Where $COV_{f(x,y)}$ is the phenotypic covariance between characteristics x and y, $\sigma^2_{f(x)}$ and $\sigma^2_{f(y)}$ are the phenotypic variances for characteristics x and y, respectively.

Genetic correlations (r_g) between the pairs of the characteristics were estimated as (Falconer, 1989):

$$r_g = \frac{COV_{f(x,y)}}{\sqrt{\sigma^2_{f(x)}\sigma^2_{f(y)}}}$$

Where $COV_{f(x,y)}$ is the genetic covariance between characteristics x and y, $\sigma^2_{f(x)}$ and $\sigma^2_{f(y)}$ are the additive genetic variances of characteristics x and y, respectively.

RESULTS AND DISCUSSION

Growth variations

Averages, clonal ranges, and coefficient of variations were given for the characteristics and orchards in Table 2. The oldest seed orchard (SO3) had the highest growth performance for the characteristics, while they were the lowest in the youngest seed orchard (SO2). However, SO1 showed the highest annual increments for the characteristics except of tree height of SO3 (Table 2). This may be affected by many factors (Eriksson *et al*., 1973). Kang (2001) reported that young seed orchards were more variable than that of older. Age was an important factor in growth and reproductive performances in seed orchards of Scots pine (Bilir *et al*., 2006).

Table 2. Averages (x̄), clonal ranges and coefficient of variation (CV%) for the characteristics and orchards.

	SO1			SO2			SO3		
	x̄*	**Range**	**CV**	**x̄**	**range**	**CV**	**x̄**	**range**	**CV**
TH (m)	6.45[b]	5.38-8.05	1.35	5.78[a]	4.89-6.63	1.40	9.30[c]	7.26-10.93	0.97
D_0 (cm)	25.63[b]	22.14-30.18	1.41	24.50[a]	16.32-29.48	1.59	31.91[c]	23.64-39.56	1.11
$d_{1.30}$ (cm)	22.42[b]	19.32-27.26	1.52	20.26[a]	13.88-25.38	1.68	27.13[c]	21.04-34.74	1.16
CD (m)	5.91[b]	4.24-7.76	1.71	5.00[a]	3.55-6.46	1.94	7.09[c]	5.26-8.79	1.27

**; Same letters are not significantly different among orchards.*

There were high differences among clones within orchard for the characteristics. For example, the differences were about 50% (5.38 m, 8.05 m) in SO1, 36% in SO2 and 51% in SO3 for tree height which had the lowest coefficient variations in all the orchards. There could many biotic and a-biotic factors impact on the performances and variation such as age, orchard location and genetic structures of clones (Eriksson *et al*., 1973; Bilir *et al*., 2006; Kang and Bilir, 2021). Clones were more heterogenous for crown diameter based on coefficient variations (Table 2, Figure 3). The seed orchards were established at 8m x 8 m spacing, larger than a common plantation. The variation could change in the future by competition among ramets. The results were well accordance with coefficient of variations of the characteristics in mother populations of the seed orchards. Coefficients of variations were the lowest for tree height and the highest for crown diameter in mother populations (Bilir *et al.*, 2023). However, variations among mother trees of base populations (Bilir *et al*., 2023) were higher than among clones of seed orchard populations. It could be because of management practices in the orchards. The growth variations among clones were also reported in seed orchards of different forest tree species (i.e., Varol *et al.*, 2017; Dutkuner *et al*., 2008; Bilir *et al.*, 2006), and among individuals in plantations (i.e., Kartal and Bilir, 2022) and in a seed orchard (Ertekin, 2006) of Anatolian black pine. The variation could be balanced by different management practices such as pruning. The results indicated importance of individual selection than mass selection in establishment of seed orchards. Ramets within clone showed also large differences for the characteristics. For instance, individual ramet growths were ranged from 3.92 m to 8.43 m for tree height, varied between 20.70 cm and 31.10 cm for diameter at base in first clone of SO2. They could be related to where the cuttings taken in mother tree. However, many a-biotic and biotic factors could effect on variations of growth performances (e.g., Bilir *et al*., 2018; Yazici, 2018; Yazici and Turan, 2016).

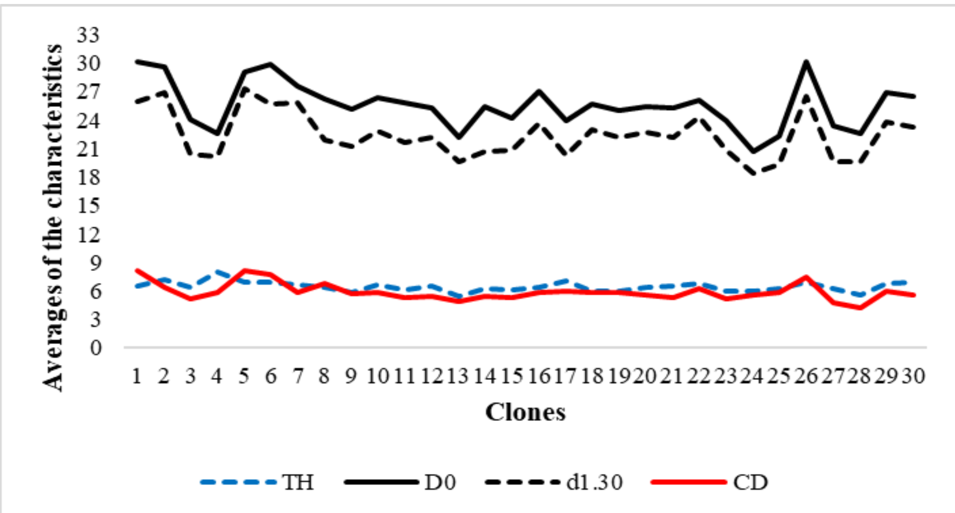

Figure 3. Clonal averages of SO1 for the characteristics

There were significant (p≤0.05) differences among clones within orchard except of tree heights of SO1 and SO2, and among the seed orchards for the characteristics based on results of analysis of variance. Tree height was more homogeneous than the other characteristics according to the Duncan's multiple range tests and coefficient of variations in each orchard (Table 2). The differences indicated different management practices for each orchard in growth characteristics. The variation could be used for different purposes such as selection of superior clone/ramet in seed orchards emphasized by Imren *et al.* (2021) or balanced by different management practices.

Correlations

Results of correlation analysis indicated positive and significant (p≤0.05), phenotypic and genotypic relations among the pairs of the characteristics in the orchards (Table 3). Similar trends were also reported in seed orchards of different forest tree species (e.g., Bhumibhamon, 1978; Dutkuner *et al*., 2008; Bilir *et al.*, 2006 and 2008). The relations emphasized that less characteristics could be used in management of orchards, and in future studies.

Table 3. Phenotypic correlations (below diagonal) and genetic correlations (above diagonal) among the characteristics for the orchards

	r_g/r_p	TH	D_0	$d_{1.30}$	CD
SO1			.429*	.468**	.468**
SO2	**TH**	-	.722**	.727**	.740**
SO3			.712**	.684**	.609**
SO1		.395**		.952**	.771**
SO2	**D_0**	.700**	-	.947**	.764**
SO3		.575**		.914**	.740**
SO1		.395**	.928**	-	.759**
SO2	**$d_{1.30}$**	.740**	.922**		.799**
SO3		.627**	.868**		.690**
SO1		.352**	.681**	.727**	
SO2	**CD**	·726**	.756**	.785**	-
SO3		.470**	.612**	.633**	

**; Correlation is significant at the 0.05 level, **; Correlation is significant at the 0.01 level.*

The phenotypic and genotypic relations (Table 3) showed easy character could be used in selection of mother trees for establishment and management practices of seed orchards by estimation of heritability for the characteristics.

CONCLUSIONS

Variation among ramets within clone emphasized importance of place where the cuttings taken from mother tree. It could be important in establishment stage of seed orchard. Variation could be a guide for selection of superior clone or ramets for second generation establishment of seed orchards for managers. Management strategies should be planned for each seed orchard. Significant phenotypic and genotypic relations among the pairs of the characteristics could be used in establishment and management practices of seed orchards. Future studies should be carried out by long-term data and different characteristics such as stem straight to produce better quality wood product.

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POPULATION DYNAMICS IN THE MOUNTAINOUS AREAS OF MONTENEGRO: ECOLOGICAL TRANSITIONS AND DEMOGRAPHIC TRANSFORMATIONS IN PLJEVLJA SINCE THE BEGINNING OF THE 21ST CENTURY

SUMMARY

The paper deals with the demographic development of the northernmost municipality in Montenegro, with a special emphasis on migration trends, which have intensified since the second half of the last century. The municipality of Pljevlja is specific in this regard because it had the highest growth in emigration since the beginning of the 21st century in Montenegro, regardless of the fact that it has natural potentials, which, unfortunately, have not been adequately used, so the municipality is still classified as underdeveloped. The consequence of the weak economic development of the municipality is the continuous decline in the number of inhabitants, namely, Pljevlja's share in the total population of Montenegro decreased from 9.7% in 1961 to 4.2% in 2021. In the paper, we will look at the ecological transitions and demographic transformations of the municipality. By analyzing the demographic parameters, we will point out the consequences of migration, which are directly reflected in the further demographic development of the municipality. Analysis of the overall movement of the population (natural movement and migration), the age-gender structure of the municipality's population and rural population size will prove the set hypotheses.

Keywords: economy, ecology, demographic development, emigration, population structure, depopulation.

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INTRODUCTION

The correlation between human development and human mobility is a matter of intense debate and academic deliberation. A commonly held belief asserts that global migration primarily stems from disparities in wealth and developmental stages across regions. This perspective is mirrored in policy frameworks and media narratives, wherein the lack of development and the associated issues of poverty and conflict are generally attributed as significant, if not the primary, drivers for migration from less affluent to more prosperous nations, as well as for rural-to-urban migration within developing countries. Consequently, the mitigation of such disparities through the enhancement of economic growth in less privileged nations is often regarded as the most potent approach to mitigate or address the phenomenon of international migration (Böhning and Schloeter-Paredes, 1994; De Haas, 2007).

Depopulation and deagrarisation appeared as the main limiting factors for rural development in Montenegro, and thus for the Municipality of Pljevlja. These phenomena particularly came to the fore after the Second World War. Industrialization has caused migration from rural areas to the administrative and economic centres (Despotovic *et al.,* 2015).

The population matters and corresponding policies have witnessed significant transformations from the 20th to the 21st century. The swift expansion of the global population, which seemed uncontrollable in the latter half of the 20th century and was prominently addressed in population policies, has currently shown a deceleration in its annual growth rates. Undeniably, while the yearly surge in population numbers remains noteworthy, an average of 87 million individuals have been incrementally included in the global populace each year from 1981 to 2020 (United Nations, 2022).

The possibility of achieving zero population growth and reaching the peak population might emerge before the conclusion of this century, albeit this notion is presently under discussion (O'Sullivan, 2022). Despite rapid population growth still occurring in sub-Saharan Africa and some other regions (mainly a few countries in the Middle East and parts of South Asia, e.g., Yemen, Afghanistan, Pakistan), most of the world's population is now experiencing low fertility and, consequently, population aging (United Nations, 2022; Goldstone & May, 2022).

The global landscape is progressively undergoing urbanization. Moreover, an array of novel challenges has surfaced; encompassing matters like climate change, emerging pandemics, global migration, and the movement of refugees (Goldstone & May, 2022).

In addition to altering the emphasis, there has been a transformation in the broader formulation of population policies, transitioning from interventions rooted in demographics to the acknowledgment and promotion of reproductive rights (Bernstein *et al.*, 2022).

The interplay between demographic processes and the developmental stage of a specific region exhibits a causal relationship. Within this intricate web of interrelations, the needs and opportunities of the populace exert an influence on spatial advancement. Conversely, the inherent and ongoing functional traits of a given space exert an impact on shaping the trajectories of demographic processes. Among the array of contemporary demographic challenges, the uneven spatial dispersion of population stands out prominently. In the course of demographic or other types of polarization, the existence of one or more focal points of population or functions becomes imperative. More often than not, the capital city assumes the role of a dominant pole, steering the development of the entire territory. Frequently, the magnetic allure and potency of the capital outpace the capacities of the rest of the nation, resulting in pronounced imbalances in population and functionality. This resultant scenario not only exacerbates the propagation of adverse population trends but also engenders fresh economic and organizational predicaments (Filipovic *et al*., 2022).

The municipality of Pljevlja is located in the northernmost part of Montenegro, with an area of 1,346 km², covering 9% of the country's territory and is the third largest municipality in Montenegro (MONSTAT, 2021). The average altitude of the municipality ranges from 1,000 to 1,200 m. An important characteristic of the municipality is its peripheral position in relation to the rest of the country, as evidenced by the fact that it is bypassed by all international road routes, and even internal roads and the main tourist routes in the country. Such position had a weighty impact on the economic development of the municipality, regardless of significant natural potentials and mineral resources, and it caused specific demographic trends, especially in the last decades of the 20^{th} and the beginning of this century.

During the former SFR Yugoslavia, industrial development began in Pljevlja, which was one of the most underdeveloped municipalities in Montenegro. First, in 1953, the "Suplja Stijena" lead and zinc flotation mine was opened, a coal mine, then the agricultural enterprise PTK Pljevlja, the transport-tourism company "Prevoz" - Pljevlja, the construction company "Gradjevinar", the knitwear and clothing industry "Tara" and forest-industrial plant "Velimir Jakic". For a short period of time (1975-1978), a cement factory worked as well, but was closed due to the environmental problems it caused. In 1982, the most important economic facility in the municipality, Thermal Power Plant Pljevlja, began operating, which today, along with the coal mine, is the sole carrier of economic development in the municipality.

The industrial development of the municipality in the second half of the last century, caused large-scale migration from rural areas to the city of Pljevlja thanks to the substantial demand for labor, which resulted in a significant decrease in the number of residents in rural areas on one hand, and a high growth in the number of residents in the city on the other. Regardless of the major industrial and economic development, there were still large-scale migrations out of the municipality, which the rates of natural increase could not compensate for, thus, since the 80s of the last century there has been a greater decrease in the number of inhabitants.

Since the second half of the last century, the population in the municipality has decreased from 46,677 in 1961 to 35,806 in 2003. In the period 1981-2003, the intensity of population decline has increased significantly compared to the previous period (1961-1981). By comparing the data for the rural and urban population, it is evident that the decrease in the population of the municipality is mainly a consequence of the decrease in the rural population from 35,200 to 14,448, while in the same period (1961-2003) the number of the urban population increased from 11,477 to 21,741. From the data on the number of inhabitants of rural settlements, the urban settlements (the city of Pljevlja and Gradac) and the municipality as a whole, it is easy to conclude that internal rural-urban migration was significantly more intense than emigration outside the borders of the municipality, in which the municipality lost 8,871 inhabitants, because, in the same period, rural settlements lost 20,752 inhabitants, while urban settlements gained 10,264 inhabitants.

MATERIAL AND METHODS

Pljevlja is a city and seat of the municipality of the same name in Montenegro. It is located in the north of the country (Figure 1) in the center of the spacious Pljeval basin at the foot of the Golubinja hill. It lies on three rivers: Breznica, Ćehotini and Vezišnica. The city is the center of the energy potential of Montenegro, an important industrial, cultural and educational center.

Figure 1. Location of Pljevlja - Map showing major population centers in Montenegro as well as parts of surrounding countries and the Adriatic Sea. (Source: Google Earth; The World Factbook Washington, DC: Central Intelligence Agency, 2021)

While scrutinizing migration patterns and their ramifications in the Pljevlja municipality, conventional demographic techniques were employed. This encompassed analysis, synthesis, and the comparative approach to unveil the socio-economic dynamics of the region and the prevailing natural and environmental factors. Statistical indicators were presented and manipulated using mathematical and statistical methodologies. Microsoft Excel was utilized for generating tables and graphs, supplemented by a comprehensive examination of both international and domestic literature that delved into the intricacies of migration's causes and consequences.

We applied a SWOT analysis as a strategic planning tool to assess the internal and external factors affecting situation in the studied region. It provides a structured framework for evaluating the strengths, weaknesses, opportunities, and threats associated with a particular context. The primary purpose of conducting a SWOT analysis was to gain a comprehensive understanding of the current state of affairs, which can then guide decision-making and strategy development. It provides a concise overview of the key aspects that will be explored in more detail.

Based on the analyzed causes of intensive emigration from the municipality of Pljevlja, as well as the consequences it caused, the paper will prove the following hypotheses:

- that as a result of increased emigration, there was a significant decrease in the number of inhabitants in the municipality, especially in rural areas;
- that the economic collapse of the municipality since the 90s of the last century significantly strengthened emigration outside the municipality;
- that rural-urban migration movements, which were particularly intense in the second half of the last century in the municipality, gained intensity at the end of the 20th and the beginning of the 21st century as a consequence of the economic decline and bad ecological situation in the municipality;
- that Podgorica and coastal municipalities are the most attractive for migrants;
- that, due to the intensive emigration from the municipality as a whole, and especially from the rural area, there were accelerated changes in the age-gender structure of the population;
- that as a result of emigration there was a demographic fragmentation of rural settlements.

Economic characteristics of the municipality today

The political events in the 90s of the last century, leading to the disintegration of the former SFR Yugoslavia, which included the former SR Montenegro, caused major changes both in the economic sphere and in other segments, which was reflected to a large extent in the demographic development of the municipality. The economic sanctions to which the state was exposed at the time caused tectonic changes in the economy, which was in crisis for many years, resulting in the shutdown of a large number of industrial companies in Montenegro, which certainly did not bypass any of the less developed municipalities. Along with the decline of industrial companies in the so-called period of transition went the privatization of companies as well, which caused a rather chaotic situation, because capacity reductions and massive layoffs followed. As a result of all that, in the municipality of Pljevlja, out of a dozen economic entities in which, according to data from 1984, 14,000 residents worked, only the Pljevlja Coal Mine and the Thermal Power Plant are in operation today. Since then, Prevoz Pljevlja, Monter, Trikotaza, many agricultural enterprises, Cementara, Tara, Sloga, Ciglana, Gradjevinar and 1. Decembar have all been shut down. The unique Sumarstvo was divided. Some of the listed companies were given more modern names after privatization, such as Optel-Vektra, Castelo-Montenegro, Monterkod. New company names did not mean new production capacities, on the contrary" (https://tvpljevlja.me/). In the meantime, due to the closure of companies and privatization, which necessarily meant a reduction in the number of jobs, in 2020 the number of employees was 5,063 (https://tvpljevlja.me/), which is a little more than 1/3 of the employees from 80s of the last century.

A significant surplus of employees had been solved mainly by regular or early retirement, so the municipality came to a situation where it has a large number of retirees, 6,691 (https://tvpljevlja.me/), which exceeds the number of workers, presenting an additional burden on employees and their earnings, if we add to that a significant number of unemployed persons (over 3,100) and other dependents. These trends in economic development in the municipality also determined the course of demographic trends to a significant extent, which has been clearly reflected since the 80s of the last century, first in the population decline, the rates of natural migration and finally in significant changes in the age structure of the population.

Environmental characteristics of the Municipality

Pljevlja is the municipality in Montenegro whose water, air and soil are the most polluted. Moreover, according to the level of pollution, Pljevlja is the European record holder. One of the biggest polluters of Pljevlja, together with the Coal Mine, is the Pljevlja Thermal Power Plant, which was put into operation in the early eighties of the last century. The plant is the main producer of industrial waste. The total annual production of waste for the territory of Pljevlja amounts to about 571 thousand tons of waste, of which 280-300 thousand tons are ash, created by burning coal as an energy fuel for power plants. In 30 years of operation, the Thermal Power Plant burned about 35 million tons of coal, of which it deposited 12 million tons of ash and slag waste. The rest of the waste consists of waste oil, mazut, waste paint, sludge from thermal power plant and other waste. Huge quantities of slag and furnace dust are not classified as hazardous waste, but require special handling. Numerous analyzes show an increased content of metals, fluorine and boron in relation to the maximum allowed concentrations. Elevated values of radioactivity were also recorded, but they were below the maximum permissible limits. The Center for Ecotoxicological Research performed a characterization of the ash and it is classified as hazardous waste due to its high pH value, which is a parameter for determining the quality of drinking water, as well as surface water (Zagadjenje u opstini Pljevlja, Slucaj Termoelektrane, 2014).

One of the biggest environmental problems in Pljevlja is the high degree of air pollution with floating particles. The measured concentrations of pollutants at the measuring point in the urban part of Pljevlja mostly reflect the influence of local sources, primarily collective boiler rooms. The state of the environment in that northern municipality is a consequence of the development of the economy, the exploitation of natural resources, the construction of settlements, roads, and other facilities. The analysis of its current state shows that mining and electricity production have a dominant influence, leading to large-scale degradation, pollution and changes in the configuration of the terrain and landscape. All of this causes major pollution of the air, surface and underground water, soil degradation and pollution, accumulation of waste, deterioration of the population's health, but also the destruction of flora and fauna in that area. Conducting a strategic environmental impact assessment ensures the compliance of the activities defined by the plan with the applicable legal regulations and state planning documents in Montenegro. It assessed possible negative impacts on the environment. The Spatial Plan of Montenegro and the National Strategy of Sustainable Development defined the preservation of the quality of the environment and the improvement of natural values, the uniqueness of the space, cultural and natural heritage of Montenegro, as well as general goals in the field of environmental protection (Izvjestaj o strateskoj procjeni uticaja na zivotnu sredinu za PUP Pljevlja, 2018.).

In Pljevlja, there are about 1,100 different individual boiler rooms within family houses, the power of which are approximately known and ranges from 20-50 kW for about 1,050 rooms to 50-100 kW for about 50 rooms, with an estimated annual consumption of about 8,000 tons of coal. It is estimated that there are about 2,500 individual fireplaces that use a stove, and for an average consumption of 3 tons of coal per household, the annual consumption amounts to 7,500 tons of coal. Furthermore, it is important to note that a significant number of households have switched to heating using pellets as fuel, but the number of these fireplaces is not yet known. Due to the high price of coal, many households use wood as fuel, for which there is no data on the amount consumed (source: Municipality of Pljevlja).

RESULTS AND DISCUSSION

In order to provide a snapshot of the situation, helping the audience understand the broader context and issues at hand we applied SWOT analyses in some segments with the idea to analyze complex information into a few key points, making it easier to grasp the main factors influencing the subject. The

SWOT framework prompts strategic thinking by categorizing factors into internal (strengths and weaknesses) and external (opportunities and threats) aspects. The insights gained from the SWOT analysis can inform decision-making processes by identifying areas for improvement, potential growth, and potential challenges.

The SWOT Analysis for Economic Characteristics and Environmental Characteristics of the Municipality of Pljevlja are presented in the following presentation.

SWOT Analysis for Economic Characteristics of the Municipality of Pljevlja

Strengths: **Resource Utilization:** Historically, the municipality had a diversified industrial base with several active economic entities, including the Pljevlja Coal Mine and Thermal Power Plant. **Industrial Legacy:** The past presence of various industries, including manufacturing and agriculture, highlights the region's potential for economic development. **Historical Resilience:** The municipality survived the tumultuous political and economic events of the 90s, displaying a degree of resilience in adapting to changes.	**Weaknesses:** **Industrial Decline:** The disintegration of the former Yugoslavia and subsequent economic sanctions led to the closure of numerous industries, resulting in a loss of jobs and economic vibrancy. **Lack of Diversification:** Over-reliance on a few key industries, such as coal and power, has left the municipality vulnerable to economic shocks. **Workforce Imbalance:** The closure of companies and privatization efforts has left the municipality with a significant imbalance between retirees, unemployed individuals, and active workers.
Opportunities: **Rejuvenation Efforts:** The decline of former industries provides an opportunity to explore new sectors, fostering economic diversification and growth. **Investment Attraction:** With proper strategies and incentives, the municipality can attract investment in emerging industries, creating new job opportunities. **Re-skilling Workforce:** Reskilling and upskilling initiatives can equip the workforce with new skills required for emerging industries, promoting employment and adaptability.	**Threats:** **Economic Instability:** A heavy reliance on a shrinking industrial base, coupled with unemployment and workforce imbalances, poses a threat to the economic stability of the municipality. **Population Exodus:** The lack of job prospects and economic opportunities may lead to a continued outmigration of working-age population, aggravating demographic challenges. **Depopulation Spiral:** Economic decline can lead to reduced services, infrastructure deterioration, and a further decline in the quality of life, potentially creating a self-reinforcing cycle of depopulation.

The economic characteristics of the Municipality of Pljevlja exhibit a complex interplay of strengths, weaknesses, opportunities, and threats. While the decline of key industries presents challenges, it also opens doors for renewal and diversification. Strategic efforts to attract investment, re-skill the workforce, and diversify the economic base could help counteract the threats of depopulation and economic instability, paving the way for a more resilient and prosperous future.

SWOT Analysis for Environmental Characteristics of the Municipality of Pljevlja

Strengths: **Awareness:** The assessment clearly highlights the severe environmental issues faced by the municipality, creating awareness for potential action. **Detailed Data:** The analysis provides specific data on pollutants, waste production, and environmental impact, offering a comprehensive understanding of the situation. **Legal Framework:** The existence of legal regulations and state planning documents for environmental protection provides a foundation for addressing the issues.	**Weaknesses:** **Pollution Magnitude:** Pljevlja is recognized as a European record holder for pollution, indicating the seriousness of the problem. **Limited Clean Energy Use:** The prevalence of coal, oil, and other pollutants in heating methods suggests a reliance on non-renewable energy sources, contributing to pollution. **Lack of Consumption Data:** Insufficient data on wood consumption for heating complicates the assessment of its impact on pollution.
Opportunities: **Transition to Cleaner Energy:** The shift towards using pellets as a fuel source presents an opportunity to reduce pollution and improve air quality. **Strategic Environmental Impact Assessment:** Conducting a comprehensive assessment allows for identifying potential negative impacts and addressing them before implementation. **National Strategies:** Alignment with the National Strategy of Sustainable Development encourages efforts to preserve environmental quality and improve natural values.	**Threats:** **Health Implications:** Pollution contributes to deteriorating population health, which can lead to increased healthcare costs and decreased quality of life. **Economic Impact:** Ongoing pollution may discourage tourism and investments due to concerns over environmental conditions. **Flora and Fauna:** Environmental degradation poses a threat to local biodiversity, affecting both plant and animal life in the area.

The SWOT analysis conducted on the environmental characteristics of the Municipality of Pljevlja reveals a complex web of challenges and opportunities that require immediate attention. The strengths lie in the heightened awareness generated by the assessment, shining a light on the pressing environmental concerns that demand action. The presence of detailed data and a legal framework for environmental protection serves as a foundation for addressing these issues effectively.

However, the weaknesses underscore the gravity of the situation. Pljevlja's reputation as a European record holder for pollution emphasizes the urgent need for intervention. Reliance on non-renewable energy sources and the lack of consumption data for certain fuels only amplify the challenges at hand.

The opportunities present a ray of hope amidst these challenges. The potential transition to cleaner energy sources, such as pellets, offers a chance to mitigate pollution and enhance air quality. The significance of conducting strategic environmental impact assessments cannot be underestimated, allowing proactive identification and mitigation of negative consequences. Aligning with national sustainable development strategies further reinforces the commitment to preserving environmental quality and natural values.

Nonetheless, threats loom large, warning of the consequences of inaction. Pollution's adverse effects on public health, including increased healthcare costs and diminished quality of life, are a stark reminder of the urgency. Economic repercussions, including reduced tourism and investment due to environmental concerns, cannot be ignored. Equally important is the threat posed to local flora and fauna, underscoring the delicate balance that has been disrupted by environmental degradation.

In sum, this SWOT analysis illuminates the intricate interplay of forces shaping Pljevlja's environmental landscape. It underscores the need for a collective effort to harness strengths, address weaknesses, capitalize on opportunities, and counter threats. Only through a comprehensive and coordinated approach can the municipality pave the way toward a more sustainable and resilient future, where both the environment and its inhabitants thrive.

Characteristics of demographic development since the beginning of the 20th century

The trend of population decline that intensified since the 1980s continued at the beginning of this century, with a slightly lower intensity (table 1). According to the population estimate data for 2021[3], there were 25,917 inhabitants in the municipality of Pljevlja, which indicates the presence of emigration and the continuation of the growing trend of negative rates of natural increase. In comparison to the previous period, a slightly lower intensity of the decline in the rural population, but also a major decline in the urban population are visible, which is an indicator that the bad economic situation in the municipality and city caused the intensification of emigration from the urban area to other municipalities and beyond the borders of the country. As seen from the data in the table, the rural population dropped to only 31.7% compared to the second half of the last century (1961), thus the rural areas are already demographically emptied with mostly elderly households, causing lower intensity of population decline.

Mobility is one of the basic characteristics of the population. Migration has been present in the territory of the municipality of Pljevlja since ancient times.

[3] Monstat, Table: Estimated number of inhabitants in the middle of 2021.

Depending on the historical, political and economic conditions, sometimes there was stronger emigration, sometimes immigration.

Table 1: Population trends of the municipality of Pljevlja in the period 1961-2011

		1961	1981		2003		2011	
	Settlement	Number	Number	Index	Number	Index	Number	Index
Urban	Gradac	925	637	68,9	364	57,1	295	81
	Pljevlja	10552	17440	165,3	21377	122,6	19327	90,4
Total urban		11477	18077	157,5	21741	120,3	19622	90,3
Rural		35200	25239	71,7	14065	55,7	11164	79,4
Municipality		46677	43316	92,8	35806	82,7	30786	86,0

Source: Base study for the needs of PPCG Demographic Development, 2018; page 7; 26; calculations

Today's migration on the territory of the municipality is mostly economic in nature. There are fewer and fewer internal rural-urban migrations, and more and more those outside the borders of the municipality and even the country. Mostly young or younger middle-aged population leaves off for a better life because the rather bleak economic situation in the municipality does not offer them basic living conditions. Due to the impossibility of employment, they are unable to form a family which is reflected in other segments of demographic development.

Due to the lack of adequate data [4], the only way to find out the number of emigrants from the municipality is through the overall movement of the population and natural increase. The disadvantage of this method is that it is not possible to analyze the types or patterns of migration. According to the results of the recalculation (Table 2), the municipality of Pljevlja has lost 30,293 inhabitants through emigration since the second half of the last century, of which 6,287 in the period 2003-2021.

Table 2: Migration balance of the municipality of Pljevlja in the period 1961-2021

		1961-1981	1981-2003	2003-2021
Municipality Pljevlja	Total movement	-3361	-6403	-9889
	Natural increase	9955	4287	-3602
	Migration balance	-13316	-10690	-6287

Source: Base study for the needs of PPCG Demographic Development, 2018; page 16; Table - estimated population size in 2021: Statistical yearbook for 2021, recalculations

Unfortunately, our statistical office does not have records of external migration (outside the country's borders), so we also arrive at the number of

[4] The last official census in 2011 did not process migrations, and Monstat - the Public Statistical Office only has records of internal migration in Montenegro since 2008, while we do not have any official data for external migration, so it is impossible to carry out serious analyses.

emigrants since the last census in 2011 by calculation, thanks to the fact that internal migration has been recorded since 2011. By recalculating the ratio of the total movement of the municipality's population from 2011 to 2021 (-4,869), the natural increase (-2,402)[5] in that period and the migration balance of internal migration (-1,647), we arrived at the data that the total migration balance of the municipality is -2 467 inhabitants. This indicates that 820 inhabitants of the municipality of Pljevlja left the country, which means that the volume of internal migration to other municipalities in Montenegro is significantly larger. In the observed period, 2,730 inhabitants migrated to other municipalities in the country. The largest number of migrants from Pljevlja moved to the capital Podgorica (1,628) and to the coastal municipalities: Budva, Bar, Herceg Novi, Kotor, Tivat and slightly less to Ulcinj. When it comes to continental cities, Niksic and Danilovgrad were most attractive for migrants after Podgorica. Cities from the surrounding areas, that is, from the northern region of the country, to which this municipality also belongs, were least attractive to migrants (Table 3).

Table 3. Migration from the municipality of Pljevlja to other municipalities in the period 2011-2021

Registration municipality	**Deregistration municipality of Pljevlja**											
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	**2011-2021**
Andrijevica					1	2						**3**
Bar	27	25	27	29	23	24	14	36	19	18	6	**248**
Berane	4	5	2	1	3	1	1	1	2	2	3	**25**
Bijelo Polje	8	2	2	6	8	3	1	3	4	3	6	**46**
Budva	17	7	15	24	33	28	26	32	46	11	15	**254**
Cetinje			2	2					3	1		**8**
Gusinje												**0**
Danilovgrad	3	1	7	7	2	6	5	9	5	4		**49**
Herceg Novi	21	11	3	5	11	12	7	19	13	5		**107**
Kolasin			1	1		1			1		1	**5**
Kotor	3	6	2	4	9	11	14	15	13	5	8	**90**
Mojkovac			1	1	1	3	4	2			3	**15**
Niksic	6	10	5	4	6	8	10	5	6	4	2	**66**
Plav									2		1	**3**
Pluzine			1	1						1		**3**
Petnjica								1				**1**
Podgorica	138	138	164	136	122	147	155	229	194	158	47	**1628**
Rozaje				2			3	1			2	**8**
Savnik					1	2		1				**4**
Tivat	1	5	4	4	3	10	13	18	9	12	5	**84**
Tuzi										1		**1**
Ulcinj	2		3	2	1	5		2	5	1	3	**24**
Zabljak	12	5	6	3	7	3	3	5	3	9	2	**58**
Total	**242**	**215**	**245**	**232**	**231**	**266**	**256**	**379**	**325**	**235**	**104**	**2730**

Source: Monstat – Tables: Internal migration in Montenegro in: 2011, 2012, 2013, 201, 2015, 2016, 2017, 2018, 2019, 2020 and 2021

[5] Data from statistical yearbooks in 2016 and 2021

Such directions of internal migration are quite understandable because the population, as already emphasized, today mainly migrates for economic reasons, and the most economically developed part of Montenegro, besides the municipality of Podgorica, are the coastal municipalities.

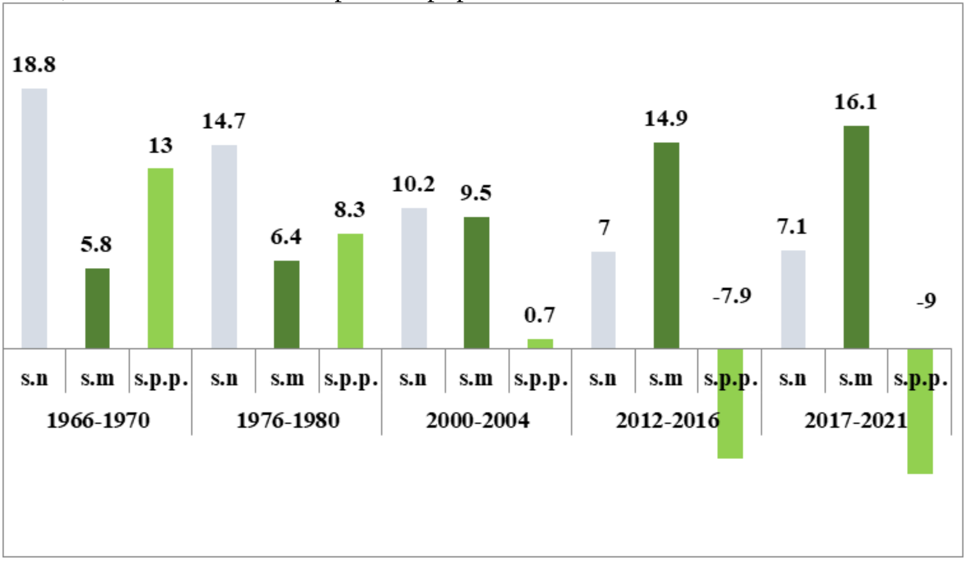
Internal migration in the opposite direction - towards the municipality of Pljevlja was significantly smaller in volume (1,083 inhabitants) in the observed period (2011-2021). The largest number of migrants towards Pljevlja is from the municipality of Podgorica (545), then from the coastal municipalities: Budva, Bar, Herceg Novi and Kotor. The continental municipalities, Niksic and Bijelo Polje had a slightly higher number of migrants towards the municipality of Pljevlja (Table 4). We do not have data on whether these are seasonal migrants (migrants who spent a part of the year in other municipalities for work), or whether they are returnees to their birthplace who spent their working life in another place, and are returning to their home as retirees, so we cannot provide detailed analyses.

Table 4. Migration from other municipalities to the municipality of Pljevlja in the period 2011-2021

Deregistration municipality	Registration municipality of Pljevlja											
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	**2011-2021**
Andrijevica					2	1						**3**
Bar		8	9	5	8	4	5	6	5	4	26	**80**
Berane	10		1	1	1			2	1	2		**18**
Bijelo Polje	8	4	6	3	6	4	3	6		3	5	**48**
Budva	3	4	3	9	7	6	5	12	10	8	41	**108**
Cetinje	3	1	1	1	2				1	1	1	**11**
Gusinje												**0**
Danilovgrad		1			1	1	1		1	1	5	**11**
Herceg Novi	3	3	8	2	5	9	2	2	3	2	12	**51**
Kolasin		1			2	1		2				**6**
Kotor	1	3	3	1		2	3	3	5	2	6	**29**
Mojkovac	1	1	3	1	1	1	1	1				**10**
Niksic	7	1	4		4	6	2	3	1	8	8	**44**
Plav	1	1	1	1				1				**5**
Pluzine											1	**1**
Petnjica												**0**
Podgorica	33	27	21	24	45	46	44	39	43	36	187	**545**
Rozaje	1					2		4	1		3	**11**
Savnik	1	1						1	1			**4**
Tivat				8				5			9	**22**
Tuzi									1		1	**2**
Ulcinj	1			1	1		2	5	1	2	5	**18**
Zabljak	4	6	8	4	4	3	1	5	4		17	**56**
Total	**77**	**62**	**68**	**61**	**89**	**86**	**69**	**97**	**78**	**69**	**327**	**1083**

Source: Monstat – Tables: Internal migration in Montenegro in: 2011, 2012, 2013, 201, 2015, 2016, 2017, 2018, 2019, 2020 and 2021

Birth rates in the municipality declined from moderate in the period 1966-1970 down to low levels in the period 2017-2021, while mortality rates in the same period rose from low to very high, resulting in negative rates of natural increase even in the first years of 21st century with a tendency of significant growth (graph). As a consequence of this trend, we observe a decrease in population in the municipality on two grounds - natural movement and emigration, which threatens further demographic development, especially in rural areas, and leads to their complete depopulation.



Graph 1: Rates of natural population movement of the municipality of Pljevlja in the period 1966-2021 (Data source: Basic study - Demographic development, page 12, Statistical yearbook for 2021)

There were significant changes in the age-gender structure of the municipality's population from the second half of last century to the end of the first decade of this century. The gender structure of the population as a whole was satisfactory in both observed periods, however, as observed in larger age groups, there is a noticeable difference in the middle-aged population group in the two observed periods. In the first period in 1961, due to the consequences of the World War II, the rate of masculinity was slightly lower (89.6), while in the second observed period it was significantly higher compared to the previous period and even higher than the rate of femininity. This is a consequence of migration movements from rural areas where, as a rule, more of the male workforce remains, while the female population moves out. This problem is present in all Montenegrin villages, which significantly affects the reproduction of the population, because in the villages, on family estates, mostly male workforce remains, which, due to the lack of female population, is unable to form a family.

Table 5. Masculinity rates of municipality of Pljevlja in 1961 and 2011

Rates of masculinity	1961				2011			
Municipality of Pljevlja	Total population	0-19	20-59	60+	Total population	0-19	20-59	60+
	95,9	103	89,6	90,9	96,4	101,7	107,8,	72,2

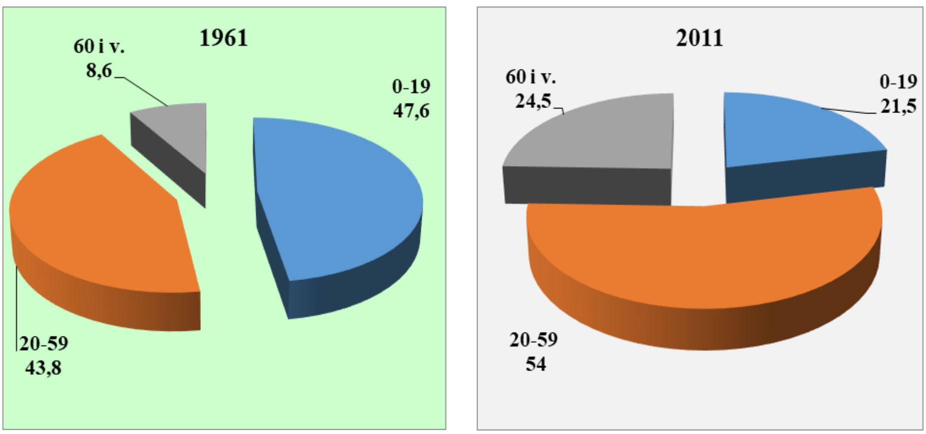
Source: Base study for the needs of PPCG Demographic Development, 2018; page 39.

Apart from gender, significant changes also occurred in the age structure of the population. In the sixties of the last century, the population of the municipality was categorized as particularly young, with as many as 47.6% young and 8.65% old. Thanks to the large emigration that affected the decrease in the birth rates and the increase in the mortality rates, resulting in negative rates of natural increase, the age structure underwent significant changes, so in 2011 the share of young people amounted to only 21.5%, while the share of old increased to 24.5%, which indicates the old demographic age of the population. It should be emphasized that the age structure of the female population of the municipality is less favorable than that of the male population, which is a consequence, as already mentioned, of the greater emigration of the female population.

Table 6. Age structure of the population of the municipality of Pljevlja in the period 1961-2011

	Gender	1961			1981			2003			2011		
		0-19	20-59	60+	0-19	20-59	60+	0-19	20-59	60+	0-19	20-59	60+
Municipality of Pljevlja	Total	47,6	43,8	8,6	33,6	54,6	11,8	24,5	52,6	22,9	21,5	54	24,5
	M	49,4	42,3	8,3	34,8	54,4	10,8	25,3	55	19,7	22,1	56,9	72,2
	F	46	45,2	8,8	32,5	54,8	12,7	23,7	50,3	26	20,9	51	27,9

Source: Base study for the needs of PPCG Demographic Development, 2018; page 50.



Graph 2: Population of the municipality of Pljevlja by larger age groups in 1961 and 2011

The fact that the municipality of Pljevlja is among the oldest municipalities in Montenegro, with an average population age of 41.8 years and an age index value of 113.9, speaks volumes that the situation in the age structure of the population is alarming, that is, it is high time to take measures of appropriate demographic-population policies, otherwise a large part of the northern region, to which the municipality of Pljevlja belongs, will lose all inhabitants in the coming decades.

Table 7. Demographic age index of population of Montenegro

Municipality	Average age	Younger than 20 in %	Younger than 40 in %	Older, 60+ in %	Index of ageing
Northern region					
Andrijevica	39,9	24,9	48,8	22,5	90,4
Berane	36,4	29,3	46,3	15,6	64
Bijelo Polje	36,1	28,6	56,4	17,4	60,8
Kolasin	40,1	23,3	48,7	22,4	96,3
Mojkovac	38,4	25,3	51,2	19,3	75,7
Plav	36	21,7	39,4	12,2	56,1
Pljevlja	41,8	21,5	45	24,5	113,9
Pluzine	43,7	20,2	41,8	29	143,6
Rozaje	31,7	35,4	64,4	11,7	33,2
Savnik	42,5	20,9	44,2	27,5	131,7
Zabljak	41,9	21,2	45,2	26,3	124
Central region					
Podgorica	35,7	27,4	57,9	15,8	57,9
Cetinje	40,3	20,9	48,9	22,3	106,6
Niksic	37,8	25,7	53,6	19,1	74,3
Danilovgrad	38,1	24	53,5	19,6	81,7
Coastal region					
Bar	37,9	25,2	52,9	18,9	75
Budva	36,5	24,5	56,1	19,3	62,8
Herceg Novi	40	22,6	48,9	21,7	96
Kotor	39,5	22,7	49,7	20,9	92,1
Tivat	38	24,2	52,8	18,5	76,4
Ulcinj	37,8	26,6	53,3	19,7	74,2

Source: Base study for the needs of PPCG Demographic Development, 2018; page 52.

In the middle of the last century, share of rural population amounted to as much as 75.4% in the total population of the municipality, and by 2011, according to the results of the last census, its share fell to 37.2%. This decline is a consequence of mass rural-urban migration caused by the industrialization of the municipality after the 1960s. Another significant consequence of this process is population fragmentation of villages. In 1961, there were no settlements without inhabitants in the municipality, while in 2011 there were 5. As can be seen from table no.8, from 1961 to 2011, the number of settlements with the smallest number of inhabitants (0-25) had the greatest growth, from 1 to 53, then the number of settlements with 26-50 inhabitants from 4 to 29 and the number of settlements with 50-100 inhabitants from 29 to 39. Therefore, the category of settlements with less than 100 inhabitants increased its share from 21.6% to 80.3%. In the same period, the number of medium-sized settlements (category of settlements 101-200 inhabitants and 201-300 inhabitants) decreased from 49 to 26, i.e. from 36 to 6, and their share from 54.1% to 20.4%.

Table 8. Population size of villages in the Pljevlja municipality in 1961 and 2011

	1961		2011	
	Number	%	Number	%
Desolate	0	0	5	3,2
0-25 inhabitants	1	0,6	53	33,8
26-50 inhabitants	4	2,5	29	18,5
51-100 inhabitants	29	18,5	39	24,8
101-200 inhabitants	49	31,2	26	16,6
201-300 inhabitants	36	22,9	6	3,8
301-500 inhabitants	23	14,6	2	1,3
501-1000 inhabitants	15	9,6	2	1,3
over 1 000	0	0	0	0

Source: Monstat- Census 2003- comparative overview of population size in 1948, 1953, 1961, 1971, 1981, 1991 and 2003, data by settlements, vol. 9; First census results in 2011

GAP, Trend, PESTEL and SWOT Analysis for the Demographic Development in Pljevlja

Based on the inputs and initial results of our research we conducted GAP, Trend, PESTEL and SWOT Analysis for the Demographic Development in Pljevlja. Findings are summarized and presented in the following chapters: GAP Analysis, Trend Analysis, PESTEL Analysis, SWOT Analysis for the Demographic Development in Pljevlja.

GAP Analysis

Goal was to analyze the demographic development of the municipality of Pljevlja and identify gaps between current trends and desired outcomes for population dynamics, economic growth, and environmental sustainability.

1.Demographic Development Gap:

Current Situation: The population of Pljevlja has been declining, particularly in rural areas, due to emigration driven by economic challenges and an ageing population. The gender and age structure of the population has shifted unfavorably, impacting future demographic trends.

Desired Outcome: Stabilize and potentially increase the population by implementing measures to attract and retain young individuals and families.

Address the gender imbalance and create a more balanced age distribution for sustainable growth.

2.Economic Diversification Gap:

Current Situation: Economic reliance on a few industries, notably mining and power generation, has led to job losses, limited opportunities, and economic stagnation. The closure of various companies and lack of innovation have hindered economic growth.

Desired Outcome: Diversify the local economy by promoting entrepreneurship, investing in new industries, and creating a business-friendly environment. Encourage innovation and skill development to generate diverse employment opportunities.

3.Environmental Sustainability Gap:

Current Situation: High levels of pollution from industrial activities, particularly the thermal power plant, have degraded the environment and impacted residents' health. The municipality's heavy reliance on resource-intensive industries contributes to environmental degradation.

Desired Outcome: Implement stricter environmental regulations; invest in pollution control technologies, and transition towards cleaner and more sustainable economic activities. Improve air and water quality to enhance residents' quality of life and attract potential newcomers.

4.Infrastructure and Technology Gap:

Current Situation: Insufficient technological advancements in the municipality have hindered economic growth and discouraged young people from staying. Limited technological infrastructure has contributed to the lack of diversified industries.

Desired Outcome: Invest in digital infrastructure, provide access to modern technologies, and offer training programs to develop a skilled workforce. Foster innovation and technological progress to attract tech-savvy individuals and industries.

5.Policy and Governance Gap:

Current Situation: Lack of effective demographic-population policies and poor governance has contributed to migration, economic decline, and environmental challenges. Fragmented policies hinder holistic solutions.

Desired Outcome: Develop comprehensive demographic-population policies that address economic, social, and environmental aspects. Enhance local governance to implement and enforce policies effectively, fostering collaboration among stakeholders.

6.Social and Cultural Gap:

Current Situation: Changing cultural values and perceptions, along with an ageing population, have contributed to migration trends. The decline in rural populations and changes in community dynamics are affecting social cohesion.

Desired Outcome: Promote the value of rural life, offer incentives for families to stay or relocate, and invest in community development. Nurture a sense of pride in local culture, history, and traditions to foster community bonds.

The GAP analysis reveals significant disparities between the current state of population dynamics, economic conditions, and environmental sustainability in Pljevlja and the desired outcomes. To bridge these gaps, the municipality must adopt a multidimensional approach that integrates economic diversification, environmental protection, infrastructure development, policy reform, and community engagement. This comprehensive strategy aims to create a more balanced and sustainable future for Pljevlja, focusing on attracting and retaining a diverse and vibrant population while ensuring economic prosperity and ecological well-being.

Trend Analysis

Demographic Trends:

Population Decline: The population of Pljevlja has shown a consistent decline over the years. From 1961 to 2021, the municipality's share in Montenegro's total population dropped from 9.7% to 4.2%.

Rural-Urban Shift: The demographic landscape has shifted from predominantly rural (75.4% rural population in 1960s) to urban dominance (37.2% rural population in 2011), resulting in rural depopulation.

Age Structure Changes: The age structure has transitioned from a particularly young population in the 1960s (47.6% young, 8.65% old) to an ageing population with reduced share of young individuals (21.5% young, 24.5% old in 2011).

Economic Trends:

Industrial Development: Industrialization efforts in the mid-20th century led to significant growth in Pljevlja, with the establishment of lead, zinc, coal, and power industries. However, economic challenges arose post-1990s due to the collapse of former Yugoslavia and economic sanctions.

Economic Decline: The economic collapse in the 1990s and the transition period led to the shutdown of several companies and industries. The municipality's workforce reduced from around 14,000 in the 1980s to 5,063 employees in 2020.

Environmental Trends:

Environmental Degradation: The intensive industrial activities, especially from the coal mine and thermal power plant, have caused significant environmental degradation. High levels of pollution have led to poor air quality, water contamination, and health concerns.

Resource Intensity: The continued reliance on coal as an energy source has contributed to substantial waste generation, including ash and slag. Pollution from these sources has contributed to environmental problems.

Migration Trends: *Internal Migration*: The industrialization phase led to rural-urban migration, causing shifts from rural areas to the city of Pljevlja. However, this internal migration was more intense than emigration outside the municipality.

Emigration: Since the second half of the 20th century, there has been significant emigration from the municipality, particularly due to the economic decline. Podgorica and coastal municipalities are the most attractive destinations for migrants.

<u>Natural Increase Trends:</u>

Decline in Birth Rates: Birth rates have declined over the years, particularly evident from the 1960s to the 2010s. The declining birth rates, coupled with increasing mortality rates, have resulted in negative rates of natural increase.

<u>Age-Gender Structure Trends:</u>

Gender Imbalance: Migration patterns have contributed to a higher gender imbalance, with more male population remaining due to work opportunities, leading to a higher masculinity rate.

Ageing Population: The age-gender structure has shifted towards an ageing population, with a significant decrease in the proportion of young individuals and an increase in the proportion of older individuals.

<u>Spatial Distribution Trends:</u>

Population Fragmentation: The population distribution has experienced fragmentation, with a decline in medium-sized settlements (101-300 inhabitants) and an increase in settlements with fewer than 100 inhabitants.

<u>Economic Diversification Trends:</u>

Dependency on Industries: Economic diversification has not been achieved, resulting in over-reliance on a few industries like mining and power generation. The closure of various companies has hindered economic growth and opportunities. These trends highlight the complex interplay between demographic changes, economic shifts, environmental degradation, and migration patterns in Pljevlja. Analyzing these trends helps identify the root causes of the challenges faced by the municipality and inform potential strategies for addressing them.

PESTEL Analysis

Taking into account the aforementioned analyses, results and previous discussions, the following PESTEL analysis was performed.

Political factors:

National Policies: Montenegro's national policies on economic development, infrastructure, and migration greatly impact the demographic changes in Pljevlja. Government decisions regarding incentives for businesses, investment, and regional development can influence migration patterns.

Local Governance: The policies and strategies of Pljevlja's local government regarding economic diversification, job creation, and infrastructure development can affect population trends. Effective governance can encourage people to stay in the area.

Economic factors:

Industrial Decline: The closure of numerous industrial companies in the municipality, including factories and mining operations, has led to job losses and decreased economic opportunities. This has contributed to migration, particularly of young and skilled workers seeking employment elsewhere.

Economic Opportunities: The lack of diverse economic opportunities, including industries beyond mining and power generation, affects the attractiveness of the municipality for residents. A lack of job prospects can drive people to migrate to more economically vibrant areas.

Sociocultural factors:

Migration Trends: Changing cultural values and perceptions about quality of life, education, and healthcare can influence migration. A preference for urban living and better amenities in other regions may encourage migration away from Pljevlja.

Ageing Population: The increasing proportion of elderly individuals in the population can impact community dynamics, healthcare services, and social support systems. Aged populations often experience lower birth rates and higher dependency ratios.

Technological factors:

Limited Technological Advancements: Insufficient technological development and innovation in the municipality can lead to reduced economic opportunities and hinder the creation of new industries. Lack of technological infrastructure might also discourage younger individuals from staying.

Environmental factors:

Pollution and Health Concerns: High levels of pollution, including air and water pollution, can lead to health problems and negatively affect residents' quality of life. Environmental degradation can make the municipality less attractive for families and young people.

Natural Resource Dependence: Economic reliance on industries like mining and power generation can degrade the environment, contributing to pollution and resource depletion. Transitioning to more sustainable economic activities could alleviate this issue.

Legal factors:

Environmental Regulations: Strict or relaxed enforcement of environmental regulations can impact industrial operations, pollution levels, and health outcomes. Stringent regulations can lead to closures or relocations of polluting industries, influencing migration patterns.

Migration Policies: National and regional migration policies can impact the ease of movement for individuals within the country. Favorable migration policies could encourage skilled individuals to stay or return to Pljevlja.

The population dynamics in the mountainous areas of Montenegro, particularly in Pljevlja, are influenced by a complex interplay of political, economic, sociocultural, technological, environmental, and legal factors. The municipality's history of industrial decline, pollution, and limited economic diversification has led to population decline and migration. To address these challenges, a holistic approach is required, including economic diversification, job creation, environmental remediation, and infrastructure development. Limiting factors for the development of rural areas are depopulation and deagrarization (Despotovic *et al.,* 2020). A comprehensive demographic-population policy should aim to retain and attract residents, especially young individuals, while balancing the needs of an ageing population.

SWOT Analysis for the Demographic Development in Pljevlja

Strengths: *Natural Resources:* Pljevlja possesses significant natural resources, including mineral deposits (lead, zinc, coal) that historically supported industrial growth. *Strategic Location:* Despite its peripheral position, Pljevlja's location offers potential for tourism, with its mountainous terrain and natural landscapes. *Historical Industrial Base:* The presence of industries like coal mining and power generation has historically provided employment opportunities. *Educational Institutions:* Presence of educational institutions can potentially contribute to human capital development.	**Weaknesses:** *Economic Dependence:* Overreliance on a few industries has made the municipality vulnerable to economic fluctuations and crises. *Environmental Degradation:* Heavy industrial activities have led to severe environmental pollution, affecting air, water, and soil quality. *Demographic Ageing:* A shift towards an ageing population with a declining birth rate poses challenges for future population sustainability. *Lack of Economic Diversification:* Closure of companies and limited economic diversification have resulted in reduced job opportunities and economic decline. *Limited Infrastructure:* Lack of modern infrastructure and transportation connections has hindered economic growth and development.
Opportunities: *Tourism Potential:* Utilizing the natural landscapes for eco-tourism and outdoor activities could attract visitors and generate income. *Renewable Energy:* Transitioning towards renewable energy sources could create new industries and job opportunities. *Environmental Remediation:* Addressing environmental degradation can improve quality of life, attract investment, and promote sustainable growth. *Education and Innovation:* Enhancing educational institutions can contribute to skilled workforce development and innovation. *Regional Collaboration:* Collaborating with neighboring municipalities and regions can lead to joint initiatives for economic development.	**Threats:** *Emigration and Brain Drain:* Ongoing emigration, especially of the younger population, can lead to a shrinking workforce and reduced economic activity. *Economic Decline:* Economic downturns or global economic shifts can exacerbate the existing economic challenges. *Unsustainable Resource Use:* Continued reliance on non-renewable resources without sustainable practices could deplete resources and worsen environmental issues. *Environmental Regulations:* Stricter environmental regulations could impose additional costs on industries and limit economic activities. *Competition from Other Regions:* Migration patterns towards more economically developed regions may persist, further depleting the workforce.

While natural resources and historical industries have been strengths, the environmental degradation and economic dependence pose significant challenges. To address these issues and leverage opportunities, a comprehensive approach is needed, including economic diversification, environmental remediation, infrastructure development, and collaboration with neighboring regions. This analysis underscores the need for sustainable strategies to ensure a balanced and prosperous demographic future for Pljevlja.

CONCLUSIONS

The paper presents the impact of economic and environmental events on population migration from Pljevlja since the beginning of the 21st century. We started from the hypothesis that the economic collapse of the municipality in the 90s of the last century and the worrying ecological situation in the municipality significantly influenced the increased emigration outside the borders of the municipality, resulting in the fact that the number of inhabitants of the municipality of Pljevlja has almost halved since the second half of the last century – to be exact, only 55.5% of the population from 1961 lives on the territory of the municipality today.

The main cause for this alteration in the number of inhabitants in the municipality is migration, which caused major negative changes in further demographic development. Emigration of the young and younger middle-aged population resulted in a sudden drop in the rate of natural increase, acquiring negative values at the century's beginning with a tendency for further drop, which has a negative impact on the reproduction not only of the rural population, (which is certainly significantly more endangered) but also of the municipality as a whole.

Since the second half of the last century, in the municipality of Pljevlja, as well as, indeed, whole Montenegro, there have been substantial changes in the relationship between the rural and urban population, i.e. the transformation of the agricultural population into a non-agricultural population, which had positive effects in the initial phase - however, today, due to the spontaneity of that process, the agricultural population was reduced to mostly elderly households.

It is about time for the state to start implementing an appropriate demographic-population policy, which would introduce measures to stop emigration, raise the birth rates, especially in the municipalities of the North and a large part of the Central region, and, ultimately, spatially redistribute the population more evenly, thus relieving the overpopulated parts of the country on the one hand (Podgorica and the Coastal region), and retaining the nowadays very questionable reproductive base in the Northern region on the other.

We stand at a pivotal juncture in the demographic trajectory of Pljevlja, a municipality rich in history, resources, and potential. The challenges that have beset us are formidable, but within these challenges are opportunity to transform municipality into an example of sustainable growth, resilience, and prosperity.

It is time for coordinated, strategic, and proactive action. The interplay of economic forces, environmental concerns, and migration patterns necessitates a comprehensive approach that transcends short-term fixes. The government/s and local authorities should work hand in hand, with foresight and determination, to drive forward a holistic agenda for Pljevlja's demographic rejuvenation.

1. Economic Diversification and Innovation: The time has come to break free from the chains of mono-industrial dependence. The government should foster an environment that encourages entrepreneurship, innovation, and diversification. Support for small and medium-sized enterprises, investment in emerging sectors like eco-tourism and renewable energy, and collaboration with educational institutions can catalyze a new era of economic vibrancy.

2. Environmental Restoration and Sustainability: The ecological degradation that Pljevlja faces cannot be ignored any longer. The government's commitment to stringent environmental regulations, investment in green technologies, and remediation efforts are paramount. Local authorities should champion projects that restore natural resources, promote responsible resource use, and create a healthier environment for our residents.

3. Educational Empowerment and Human Capital Development: Education is the cornerstone of progress. Both the Government/s and local authorities should prioritize the enhancement of educational institutions, skills training, and knowledge dissemination. Equipping youth with the skills to navigate modern challenges is not just an investment in individuals, but a catalyst for community-wide development.

4. Collaboration and Regional Synergy: Pljevlja does not exist in isolation. Collaboration with neighboring municipalities and regions to develop integrated strategies for growth is needed. Joint initiatives can harness shared resources, boost economic activity, and create a stronger collective impact.

5. Long-Term Vision and Inclusive Decision-Making: Government policies and local decisions must be grounded in long-term planning, encompassing the well-being of both present and future generations. Inclusivity in decision-making, involving residents, businesses, and civil society, will strengthen ownership and ensure that policies are reflective of our community's needs.

Transformative actions will shape Pljevlja to be the place where economic vitality, environmental stewardship, and social progress converge for the betterment of all in Montenegro and the neighboring region.

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UTILIZATION AND TRANSFER GUIDELINES OF FOREST GENETIC RESOURCES IN MOROCCO: BIOGEOGRAPHIC SYSTEM AS A STRATEGIC PROVENANCE DECISION-MAKING TOOL

SUMMARY

The assessment of Regions of Provenance (RoP) to preserve local gene pools and prevent mal-adapted genotype introduction is a relevant tool for the correct management of Forest Genetic Resources (FGR). However, there is limited knowledge of RoP in Morocco and no previous study has focused on theirs bioclimatic and physical characterization in detail. A study was carried out with the aim of characterizing RoP and analysing their bioclimatic and physical characteristics. Characterization of these areas is performed by biogeographic-based approach, where the elevation and area has been chosen as physical variables and six environmental variables was considered as bioclimatic variables (primarily related to temperature and precipitation). High-resolution gridded geospatial data and long-time-series climate dataset of a reference normal period 1960-1990 was used to analyse the homogeneity within and between RoP, through Geographic Information System (GIS). The results reveal that despite the intra-region ecological similarity of the 19 obtained RoP, some regions are relatively heterogeneous in terms of physical (elevation) and bioclimatic variable (temperature and precipitation). This finding has been particularly observed for RoP at a high altitude. Thus, in order to improve the intra-region environmental homogeneity, the interest of inclusion of elevation bands to the established RoP. The proposed biogeographic scheme can be considered a starting point for germplasm movement, and should be utilized in in combination with appropriate information on climatic suitability between donor origin and restoration site.

Keywords: Region of Provenance, Bioclimatic Indices, Seed Transfer Guideline, Local Provenance, Germplasm Movement, Seed Zones

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INTRODUCTION

Biogeography as a discipline is closely linked and often collaborates and intersects with the disciplines of the broader realm of physical geography, such as Soil Geography - Pedology, Biogeochemistry, Ecology, Climatology, Geomorphology, but also Conservation Biology, Ecology, Climatology and researchers in these fields often work together to gain a comprehensive understanding of the complex interactions between organisms and their surroundings.

Biogeography, by studying the distribution of living organisms across the Earth's surface, is closely linked with forest conservation and plays a crucial role in informing and guiding forest conservation efforts in several ways, by assessing the diversity of plant and animal species within a forest or a particular region.

Biogeographic studies provide insights into the specific habitats and ecological niches that exist within a forest, as well as helps identify endemic species, which are unique to specific regions. Biogeography research also informs forest conservation by identifying invasive species and understanding their distribution; and can help predict how climate change may affect the distribution of species within forests. It is a fundamental tool in forest conservation providing the knowledge and data needed to make informed decisions about where and how to protect and manage forests to preserve their ecological integrity and the diverse life forms they support. By understanding the distribution of species and ecosystems, conservationists can develop effective strategies to safeguard forests for current and future generations.

The forests are among the most important natural ecosystems (Bozovic *et al*., 2022) and human dependence upon forests is a multifaceted phenomenon due to the fact that forests provide a diverse stream of benefits to humans (Adam & El Tayeb, 2014; Beckley, 1998). Forest conservation, sustainable use of forest genetics resources (FGR), and sustainable management of the numerous forest functions are the main goals of monitoring programs in forest ecosystems at the national and international levels (Schwartz *et al*., 2007). The Food and Agriculture Organization (FAO) of the United Nations appealed strongly for the establishment of monitoring systems in order to improve the information on forest genetic resources (FAO, 2014). Conserving and managing genetic diversity contribute to increasing overall resilience of forest ecosystems, by fostering tree adaptive responses to environmental changes and in mitigating the effects of pests and diseases (Alfaro *et al*., 2014, Fady *et al*., 2016).

Due to its geographical position, Morocco is characterized by a wide range of bioclimate and a great floristic richness (Medail and Quezel, 1997, MEMEE, 2009). However, the natural ecosystems in Morocco and especially forests are continuously affected by degradation (Cauvin *et al*., 1997). So there is an urgent need to protect Morocco's forest genetic resources.

Restoration guidelines widely advocate using local genetic material to maximize local adaptation (Ozbey and Bilir, 2022), enhancing resistance to high temperatures and drought (Kolupaev *et al*., 2023), limiting ‘pollution’ of local gene pools and preventing outbreeding depression (Callaham, 1964, Keller *et al*., 2000, Mortlock, 2000). Recommendations to use Regions of Provenance (RoP) as a practical provenance decision-making tool to guide the movement of germplasm-movement are rooted in scientific literature (Brown and Marshall, 1995, McKay *et al*., 2005). Several authors indicate that Regions of Provenance is a legal instrument and a compulsory tool for the management of forest genetic resources (Camerano *et al*., 2012, Marchi *et al*., 2016). However, there is limited knowledge of Regions of Provenance in Morocco and no previous study has focused on their bioclimatic and physical characterization in detail.

Several studies have highlighted the important role of bioclimatic and physical variables, in the geographic distribution and adaptation of forest plant species (Emberger, 1955, Benabid, 2000, Horning *et al*., 2010, Bradley *et al*., 2013). Thus, the main aims of this study were to establish accurate map and to perform detailed bioclimatic and physical characterization of the Region of Provenance, by using geographical information system techniques applied to long time-series climate dataset and high-resolution gridded geospatial data. In addition, by analyzing the environmental characteristics, the purpose is to enhance the understanding of physical and bioclimatic homogeneity within and between RoP, to provide a basis for monitoring and managing Moroccan forest genetic resources properly.

MATERIAL AND METHODS

Study Area. The study area of the research consists of the kingdom of Morocco which covers 710 850 km^2 (HCP, 2018), and located between 21° and 36° North latitude and between the 1st and the 17th degree of West longitude. It is limited from the West and East by the Atlantic Ocean and Algerian borders respectively and from the North by the Mediterranean Sea and from the South by the Mauritania's borders (Figure 1).

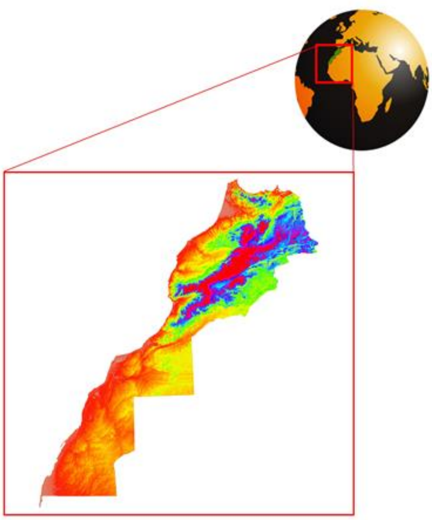


Figure 1: Map of the study area, showing the location of Morocco in the world map

Morocco is one of the most original countries in the North African region from a geographical, climatic and ecological point of view and, consequently, among the most interesting from a biological and biogeographical aspect (FAO, 2013). It contains 30 forest ecosystems covering an area of about 9 million hectares, of which 5.8 million hectares correspond to wooded forests (HCEFLCD, 2016). Its ecosystem is rich in flora, including more than 4,200 species and subspecies of vascular plants, among which, over 800 are endemic (Fennane *et al*., 1999, Fennane *et al*., 2007, Fennane *et al*., 2014).

Biogeographic system identification. As a tool commonly used in monitoring of forest genetic resources (Carolina, 1998), the Organization for Economic Cooperation and Development defined the Regions of Provenance (RoP) as a forest species or sub-species as: “the area or group of areas subject to sufficiently uniform ecological conditions in which stands or seed sources showing similar phenotypic or genetic characters Following this definition, the territory was firstly divided into relatively large Biogeographic Units (BgU) sharing great similarities in physical, topographical and forest characteristics. The resulting map was, secondly, used to delineate area subject to sufficiently uniform ecological conditions, designated as Regions of Provenance.

Lastly, the obtained RoP were classified using a coding system with two digits number. The first digit indicates the Biogeographic Units, and the second indicates the RoP within the biogeographic unit. They are written in Roman numerals system and Arabic numerals system respectively.

Characterization methodology. Taking into consideration these inferences to characterize BgU and RoP, an ensemble of eight environmental variables was used, as shown in Table 1. The Elevation and Area had been chosen as a physical variable, and six environmental variables were considered as bioclimatic variables (primarily related to temperature and precipitation).

Table 1. List of the bioclimatic and physical variables, at a spatial resolution of 1 km^2, used to characterize RoP.

Category of variables	Acronym	Unit	Spatial variable
Bioclimatic variables	AMT	°C	Annual Mean temperature
	MTWM	°C	Maximum temperature of warmest month
	MTCM	°C	Minimum temperature of coldest month
	AP	mm	Annual precipitation
	PWM	mm	Precipitation of wettest month
	PDM	mm	Precipitation of driest month
Physical variables	Elev	m	Elevation
	Ar	Km^2	Area

In addition, inter and intra-regional variations were examined based on statistical analysis of parameters related to the involved variables (mean, standard deviation, maximum and minimum values). To calculate values for the various parameters for each region, the zonal GIS techniques were performed by incorporating polygons of the RoP's vector maps and raster maps of the involved parameters.

Spatial data sourcing and analysis. As a period prior to the recent climatic disturbances, an average of 30 years of climate data from 1960-1990 with reference to normal periods was used.

The spatial climatic data were downloaded as grids at a 30 arc-seconds for about 1 km of resolution, from WorldClim dataset (Hijmans *et al.*, 2005). The WorldClim codes of the six bioclimatic variables under investigation are as follows: Annual mean temperature (BIO1), Maximum temperature of warmest month (BIO5), Minimum temperature of coldest month (BIO6), Annual precipitation (BIO12), Precipitation of wettest month (BIO13) and Precipitation of driest month (BIO14).

As physical source data, the ASTER digital elevation model (DEM) with a spatial resolution of 30 m was used, which was freely available in Land Processes Distributed Active Archive Center (LPDAAC, 2022).

Furthermore, several detailed contributions for the whole country (Boudy and Guinier, 1958, Nanson, 1995, AEFCS, 1997), served as a basis database to collect biogeographical data.

The geospatial data were implemented, processed, analyzed and mapped using the open source Geographic Information System Quantum-GIS software (QGIS version 3.22). The statistical data analysis and graphing were performed using the open source Orange Software (ORANGE version 3.33.0).

RESULTS AND DISCUSSION

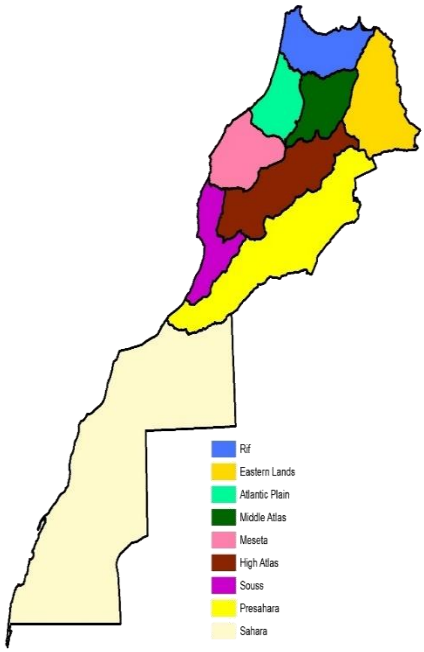
Regionalization scheme for FGR

The hierarchical organization of the Moroccan territory proposed as a biogeographic scheme for the management and conservation of forest genetic resources is presented in the Table 2.

The proposed regionalization scheme consists of the division of the country into 19 ecologically homogeneous disjointed regions for all species (Figure 2.a and 2.b). Thus, each portion of the territory is necessarily included in a RoP. This partitive method provides the advantage of defining the same Region of Provenance for all species under consideration, especially for the case of Morocco where information on population genetics and adaptation of forest species provenances are scarce.

Table 2. Regionalization scheme for FGR management and conservation in Morocco.

Biogeographic unit		**Region of provenance**	
Name	Acronym [Code]	Name	Acronym [Code]
Rif	RBgU [I]	Atlantic Rif	ARRoP [I.1]
		Western Rif	WRRoP [I.2]
		Eastern Rif	ERRoP [I.3]
Eastern Lands	ELBgU [II]	Moulouya Plain	MPRoP [II.1]
		High Plateaus	HPRoP [II.2]
Atlantic Plain	APBgU [III]	Maamora	MARoP [III.1]
		Central Plateau	CPRoP [III.2]
Middle Atlas	MABgU [IV]	Western Middle Atlas	WMARoP [IV.1]
		Eastern Middle Atlas	EMARoP [IV.2]
		Steppic Middle Atlas	SMARoP [IV.3]
Meseta	MBgU [V]	Atlantic Meseta	AMRoP [V.1]
		Continental Meseta	CMRoP [V.2]
High Atlas	HABgU [VI]	Western High Atlas	WHARoP [VI.1]
		Central High Atlas	CHARoP [VI.2]
		Eastern High Atlas	EHARoP [VI.3]
Souss	SBgU [VII]	North Souss	NSRoP [VII.1]
		South Souss	SSRoP [VII.2]
Presahara	PSBgU [VIII]	Presahara	PSRoP [VIII.1]
Sahara	MSBgU [IX]	Sahara	MSRoP [IX.1]



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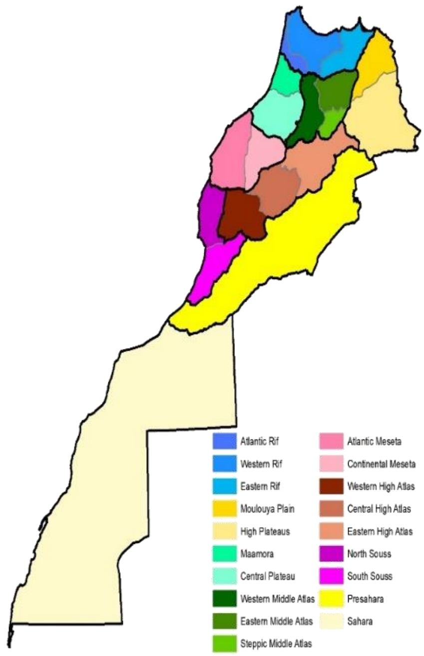

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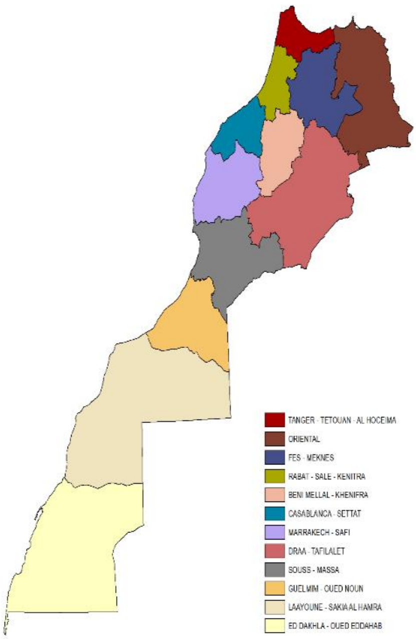


(c)

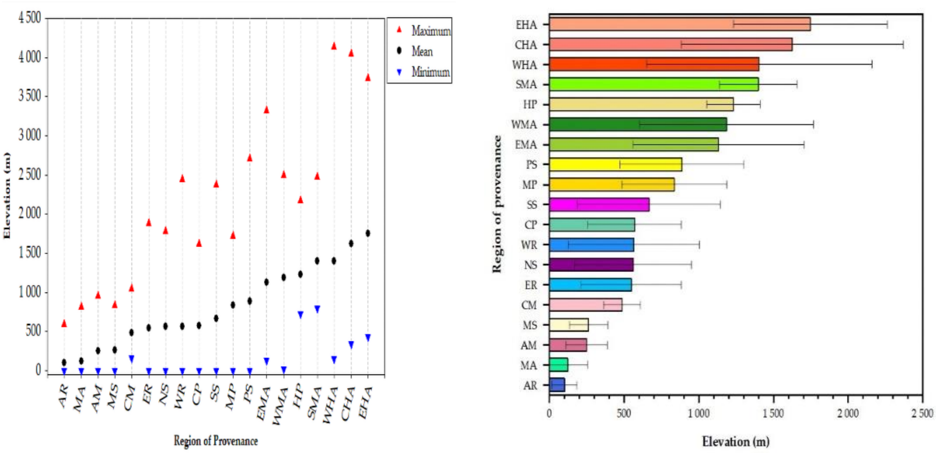
Figure 2:
a) Biogeographic Units,
b) Regions of Provenance and
c) Administrative regions maps of Morocco.

The delineated Regions of Provenance were significantly different from the Administrative Regions (AdR) shown in Figure 2.c, which had been published by the Higher Planning Commission in Morocco (HCP, 2018). Several cases were obtained according to the intersection RoP-AdR: Various Administrative Regions are fully included in a common Region of Provenance, the same RoP straddling several AdR and a single AdR covering many RoP.

Environmental characterization

Physical characteristics

The comparative analysis performed between Regions of Provenance (Figure 3), shows that the mean elevation varies from 100 m at the Atlantic Rif to 1 747 m at the Eastern High Atlas. Furthermore, the exam of altitudinal variations within RoP reveals that the minimum value 0 m has been obtained especially in the coastal regions (Atlantic Rif, Western Rif, Eastern Rif, Moulouya Plain, Maamora, Central Plateau, Atlantic Meseta, North Souss, South Souss, Presahara and Sahara RoP). The maximum value of 4 141 m was attained in Western High Atlas RoP.



(a) **(b)**

Figure 3: Fluctuation of elevation in m.
a) Within Regions of Provenance and b) Between Regions of Provenance.

The intra-region investigation (Table 3) shows relatively little variation within Region of Provenance at a low altitude. This is especially evident in the coastal regions. On the other hand, the elevation in interior regions varies widely (Western Middle Atlas, Eastern Middle Atlas, Western High Atlas, Continental Meseta, Central High Atlas, Eastern High Atlas, High Plateaus and Steppic Middle Atlas RoP).

Table 3. Physical characteristics, including area and elevation, of each Region of Provenance.

Bio-geographic unit	Region of provenance	Area		Elevation (m)		
		Surface (km^2)	Part of Morocco (%)	Lower	Upper	Mean
Rif	Atlantic Rif	6 150	0.9	0	598	100
	Western Rif	19 700	2.8	0	2 450	564
	Eastern Rif	15 400	2.2	0	1 878	547
Eastern Lands	Moulouya Plain	17 400	2.4	0	1 719	835
	High Plateaus	41 300	5.8	728	2 174	1 231
Atlantic Plain	Maamora	9 400	1.3	0	813	122
	Central Plateau	20 400	2.9	0	1 624	570
Middle Atlas	Western Middle Atlas	14 800	2.1	21	2 494	1 186
	Eastern Middle Atlas	13 700	1.9	130	3 326	1 132
	Steppic Middle Atlas	6 100	0.9	792	2 480	1 399
Meseta	Atlantic Meseta	22 900	3.2	0	952	248
	Continental Meseta	16 300	2.3	164	1 049	486
High Atlas	Western High Atlas	20 300	2.9	154	4 141	1 403
	Central High Atlas	19 100	2.7	343	4 043	1 624
	Eastern High Atlas	26 700	3.8	434	3 735	1 747
Souss	North Souss	14 400	2.0	0	1 779	560
	South Souss	17 100	2.4	0	2 378	666
Presahara	Presahara	108 000	15.2	0	2 705	886
Sahara	Sahara	301 700	42.4	0	840	261

In terms of surface area (Figure 4), it appears that Sahara and Presahara RoP are significantly the greatest (with values of 301 700 and 108 000 km^2 respectively), while Steppic Middle Atlas and Atlantic Rif RoP are noticeably smaller (with values of 6 100 and 6 150 km^2 respectively).

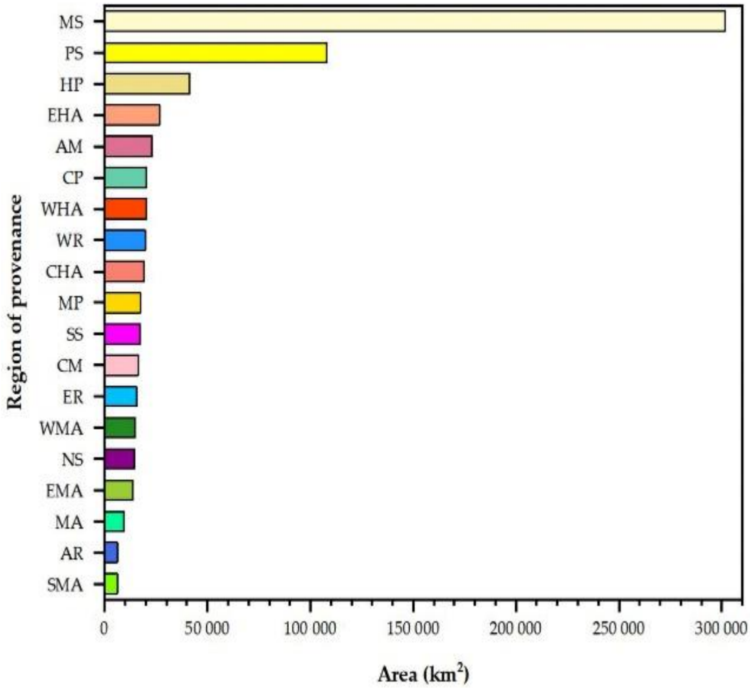


Figure 4: Area of various Regions of Provenance.

Bioclimatic characteristics

Temperature-related variables. The analyses of the temperature fluctuation within RoP (Table 4, Figure 5), indicate that:

-The annual mean temperature ranges from 0.8 to 27 °C in Morocco. The minimum of the annual mean temperature is recorded in the Western High Atlas. This region comprises the highest and the coldest segment of the entire Atlas range, including Mount Toubkal (ranging to over 4 167 m). The maximum of the annual mean temperature occurred in the Saharan Region of Provenance.

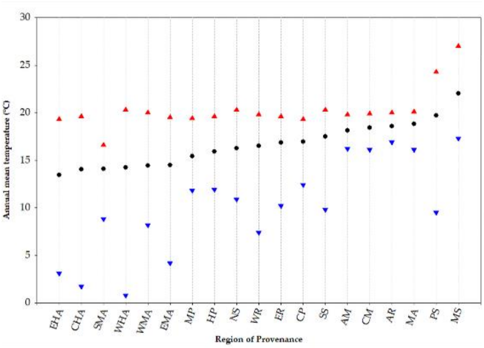
-The maximum temperature of the warmest month varies from 22 to 46.6 °C. The lowest maximum temperature of warmest month occurs in the mountain region of the Western High Atlas. The highest maximum temperature of warmest month occurs in the Presahara biogeographic unit.

-The minimum temperature of the coldest month is more than -15 °C and less than 13.6 °C. The smallest values have been found in the Central High Atlas, while the biggest values have been reached in the Sahara RoP.

As expected, the spatial pattern of temperature mean shows a large increase in the southern parts of Morocco over the Presaharan and Saharan RoP, and a significant decrease in temperature across the RoP belonging to the High and Middle Atlas, which extends over the Atlas chain.

Table 4. Characteristics of the bioclimatic variables related to temperature of RoP

Bio-geographic unit	RoP	Annual mean temperature (°C)			Max temperature of warmest month (°C)			Min temperature of coldest month (°C)		
		Lower	Upper	Mean	Lower	Upper	Mean	Lower	Upper	Mean
Rif	Atlantic Rif	16.9	20.0	18.5	28.5	36.5	33.3	4.0	9.0	6.2
	Western Rif	7.4	19.8	16.5	26.3	36.9	31.9	-6.2	8.7	4.2
	Eastern Rif	10.2	19.6	16.9	28.1	37.0	32.1	-3.1	8.6	4.4
Eastern Lands	Moulouya Plain	11.8	19.4	15.4	28.0	34.3	31.3	-2.1	8.2	2.6
	High Plateaus	11.9	19.6	15.9	31.2	39.6	34.8	-3.8	3.2	0.6
Atlantic Plain	Maamora	16.1	20.1	18.8	28.2	36.6	33.7	2.8	8.2	6.8
	Central Plateau	12.4	19.3	17.0	27.7	39.6	33.7	-2.9	7.9	3.7
Middle Atlas	Western Middle Atlas	8.2	20.0	14.4	28.8	39.6	33.6	-7.2	7.3	-0.1
	Eastern Middle Atlas	4.2	19.5	14.5	25.7	37.0	32.8	-11.1	5.9	0.2
	Steppic Middle Atlas	8.8	16.6	14.1	29.1	35.3	33.1	-6.3	1.8	-0.5
Meseta	Atlantic Meseta	16.2	19.8	18.1	27.3	38.0	31.6	1.9	9.4	6.4
	Continental Meseta	16.1	19.9	18.4	31.1	39.6	36.4	1.2	6.1	4.3
High Atlas	Western High Atlas	0.8	20.3	14.2	22.0	38.2	31.9	-14.8	6.9	-1.0
	Central High Atlas	1.7	19.6	14.1	24.6	40.1	34.6	-15.0	4.8	-2.2
	Eastern High Atlas	3.1	19.3	13.5	25.8	40.6	34.3	-13.2	4.0	-2.4
Souss	North Souss	10.9	20.3	16.2	22.4	33.7	26.8	-2.0	10.4	5.1
	South Souss	9.8	20.3	17.5	24.5	35.4	29.9	-4.9	11.8	4.9
Presahara	Presahara	9.5	24.3	19.7	25.7	46.6	39.0	-7.5	11.5	2.7
Sahara	Sahara	17.3	27.0	22.0	24.1	44.6	34.5	4.2	13.6	10.4



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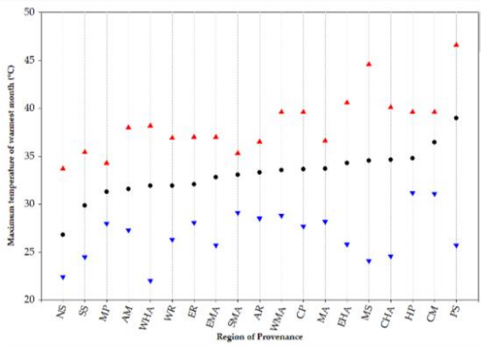

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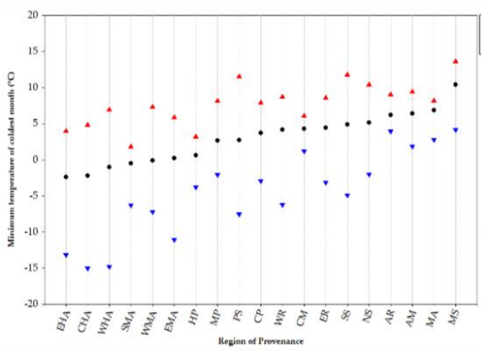
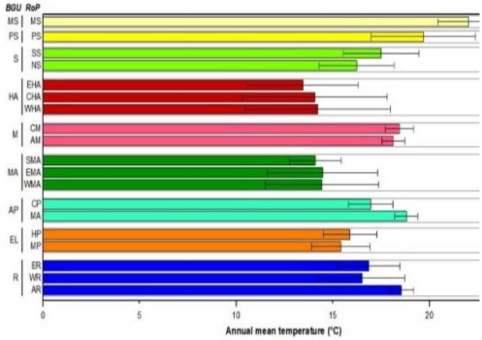


(c)

Figure 5. Temperature fluctuation within Regions of Provenance in degrees Celsius (°C). a) Annual mean temperature; b) Maximum temperature of warmest month; and c) Minimum temperature of coldest month.



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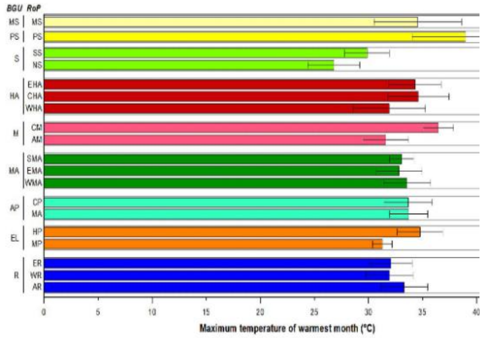

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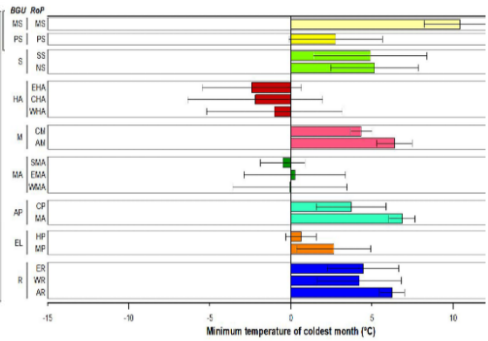


(c)

Figure 6. Temperature fluctuation between Regions of Provenance in degrees Celsius (°C). a) Annual mean temperature; b) Maximum temperature of warmest month; and c) Minimum temperature of coldest month.

On the other hand, in the Figure 6, the length of the various bars displays the mean with standard deviation of the data obtained for each RoP in terms of temperature-related variables.

The comparison between RoP shows that the annual mean temperatures ranged from 13.5°C at the Eastern High Atlas to 22°C at the Sahara. Concerning the mean maximum temperatures of the Warmest Month, the continental part of Presahara RoP registered the greatest value (39°C), while the Eastern High Atlas showed the lowest value of mean minimum temperatures, -2.4 °C, of the Coldest Month. Thus, based on the mean value by RoP, Eastern High Atlas is the coldest, whereas Presahara and Sahara are the warmest RoP. This can be explained by the effect of the high altitude on the Eastern High Atlas and the influence of the Saharan climate on the Presahara and Sahara RoP.

The mean values of bioclimatic variables for RoP over biogeographic units are relatively homogeneous. However, based on the findings of the standard deviation values, two main categories of RoP can be distinguished regarding the homogeneity of the data within the same Region of Provenance. The first category consists of the regions with low to medium altitude located in northern coasts of Morocco (e.g., Maamora, Central Plateau, Atlantic Meseta, Continental Meseta, Atlantic Rif), where the standard deviation values show a little variability in temperature. The second category includes RoP with relatively large variability, which is generally located in high altitude and/or extends across large areas (e.g., Western High Atlas, Central High Atlas, Western Middle Atlas, Eastern Middle Atlas, Presahara, and Sahara).

Precipitations-related variables. The analyses of the precipitation fluctuation within RoP (Table 5 and Figure 7), indicate that:

-The annual precipitation is more than 16 mm and less than 1182 mm. The smallest value has been found in the Sahara, while the biggest value is reached in the Western Rif RoP.

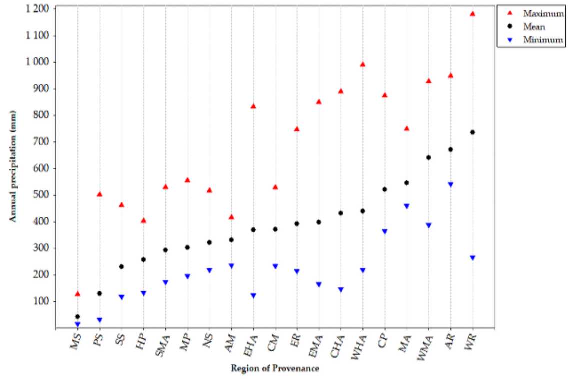
-The precipitation of the wettest month ranges from 4 to 205 mm. The minimum of precipitation of the wettest month is recorded in the Sahara RoP. The maximum of the precipitation of the wettest month occurs in Western Rif Region of Provenance.

-The precipitation of the driest month varies from 0 to 29 mm in Morocco. The lowest precipitation of driest month occurs in Atlantic Rif, Western Rif, Eastern Rif, Maamora, Central Plateau, Western Middle Atlas, Atlantic Meseta, Continental Meseta, Western High Atlas, North Souss, South Souss, Presahara and Sahara Regions of Provenance. The highest precipitation of the driest month occurs in the Eastern Middle Atlas RoP.

The spatial pattern of temperature means during the normal period 1960-1990 reveal that the mountainous RoP of the Rif and Atlas chains have received the highest amounts of precipitation, followed by the Atlantic and Eastern regions and then by the Southern regions.

Table 5. Characteristics of bioclimatic variables related to precipitation of RoP.

Bio-geographic unit	RoP	Annual precipitation (mm)			Precipitation of wettest month (mm)			Precipitation of driest month (mm)		
		Lower	Upper	Mean	Lower	Upper	Mean	Lower	Upper	Mean
Rif	Atlantic Rif	542	950	672	94	169	125	0	1	0
	Western Rif	266	1182	737	44	205	125	0	12	1
	Eastern Rif	215	747	392	35	104	62	0	9	2
Eastern Lands	Moulouya Plain	197	555	303	28	74	42	1	10	3
	High Plateaus	133	404	257	18	67	34	1	11	4
Atlantic Plain	Maamora	460	750	546	80	129	102	0	2	0
	Central Plateau	365	875	521	66	133	86	0	6	1
Middle Atlas	Western Middle Atlas	389	929	641	52	140	93	0	14	6
	Eastern Middle Atlas	165	851	397	23	102	55	1	29	6
	Steppic Middle Atlas	174	531	293	25	67	40	4	15	7
Meseta	Atlantic Meseta	236	417	331	33	81	57	0	3	0
	Continental Meseta	235	528	372	33	93	62	0	3	1
High Atlas	Western High Atlas	218	992	439	33	122	62	0	27	3
	Central High Atlas	147	890	432	25	108	62	1	27	4
	Eastern High Atlas	124	833	369	19	121	51	1	28	7
Souss	North Souss	218	518	322	36	73	53	0	4	1
	South Souss	118	463	230	27	68	40	0	4	1
Presahara	Presahara	32	502	129	9	72	23	0	9	1
Sahara	Sahara	16	128	42	4	34	10	0	1	0



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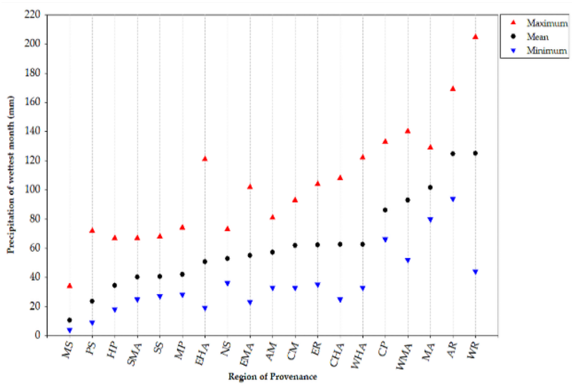

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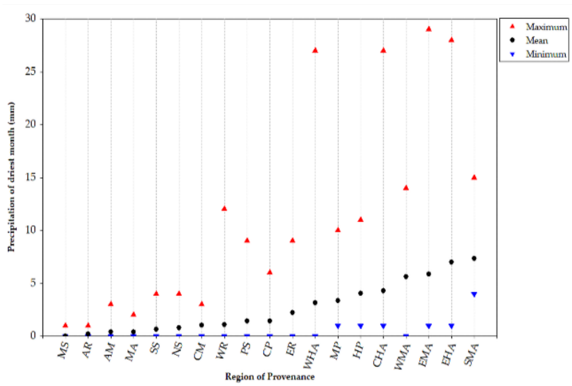
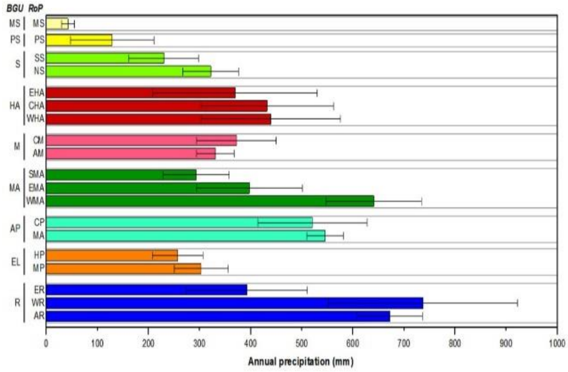


(c)

Figure 7. Precipitation fluctuation within Regions of Provenance in millimetres (mm). a) Annual precipitation; b) Precipitation of wettest month; and c) Precipitation of driest month.



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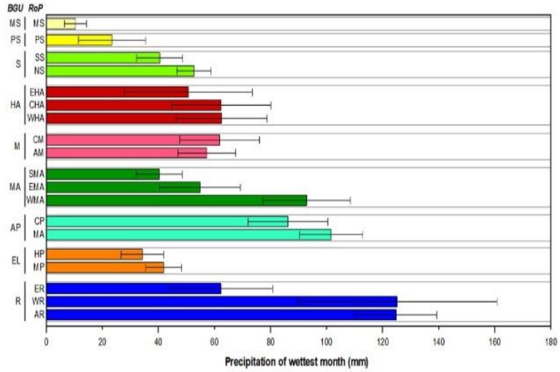

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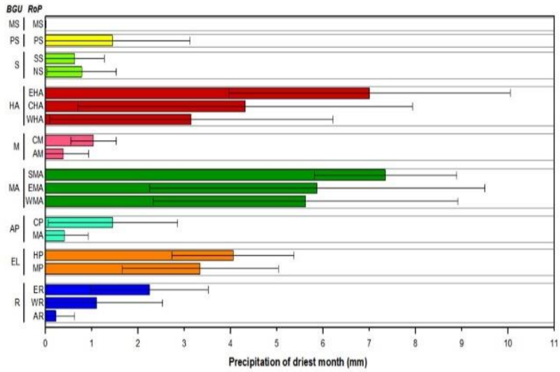

(c)

Figure 8. Precipitation fluctuation between Regions of Provenance in millimetres (mm). a) Annual precipitation; b) Precipitation of wettest month; and c) Precipitation of driest month.

At the same time, the length of the various bar in the Figure 8 displays the mean and standard deviation of the data obtained for each RoP in terms of precipitations-related variables.

Regarding the precipitations, the comparison between RoP shows that the mean annual precipitations vary between 42 mm and 737 mm in the Sahara and high mountains of the Western Rif respectively. With a notable decreasing gradient from north to south, mean precipitations of wettest month exceed 125 mm at the Western and Atlantic Rif, when the mean precipitations of driest month are less than 1 mm at Sahara, Atlantic Rif, Atlantic Meseta and Maamora RoP.

As seen in the case of temperature, two main groups of RoP can also be identified according to their intra-regional homogeneity and the precipitation means values. One group is formed by Regions of Provenance located in area without pronounced topographic features, which are characterized by little variability in precipitation (e.g. Maamora, Atlantic Meseta, and Sahara). The other group, for which the standard deviation analysis shows relatively large variability, contains the major Regions of Provenance situated in medium and high mountainous parts of the country (e.g. Western High Atlas, Central High Atlas, Eastern High Atlas, Western Middle Atlas, Eastern Middle Atlas, South Souss, Central Plateau, Continental Meseta, and Presahara).

In several countries, the RoP constitutes a valuable framework used in forestry to manage forest genetic resources (Carolina, 1998, O'Neill and Aitken, 2004, St Clair, 2014). The Regions of Provenance proposed in this study delineate areas with sufficiently uniform ecological conditions within which forest seed, cuttings and planting stock can be moved with loss of productivity and limited risk of maladaptation. It represents an essential tool to guide the choice of appropriate germplasm for forest restoration in Morocco.

On the whole, the obtained BgU presents great similarity with the structural domains of Morocco, which have been identified based on their geomorphological characteristics and geological features (Michard, 1976, Hoepffner, 1987, El Hassani, 1990, Piqué, 1994, Piqué *et al*., 2007). Furthermore, the comparison with the levels of vegetation for Moroccan flora proposed by Benabid (Benabid, 2000), shows relatively high correspondence to the Regions of Provenance presented in this study, which indicates that the method adopted to delimitate RoP allows to obtain areas that are ecologically homogeneous.

The RoP identified are significantly different from the administrative regions. Therefore, these administrative regions cannot be used as technical or regulatory instrument to invent, preserve or manage RGF. Similar results are founded by several countries adopting the partitive method to establish the regions of provenance (CEMAGREF, 2003, BFW, 2017, UKFC, 2017).

However, despite their intra-region ecological similarity, it has been shown that some RoP are relatively heterogeneous in terms of physical (elevation) and bioclimatic variables (temperature and precipitation). This finding has been particularly observed for RoP at medium and high altitude.

To improve the homogeneity of Regions of Provenances obtained in this study, the use of elevation bands within RoP would be of interest, as recommended by several authors in the case of authors' countries (Adams and Campbell, 1982, Conkle, 1997). This requires more information on the establishment of ecotypes from each RoP (survival, growth, productivity, etc.), based on reciprocal transplant studies evaluating intra-specific local adaptation.

Such information would certainly require substantial amounts of time. While waiting for this, the study proposes to adopt a provisional germplasm movement system using the proposed Regions of Provenance in conjunction with generalized elevation bands in 500-meter increments. The resulted Sub regions called "Seed Zones" are intended to be applied to all species.

To number the provisional system, this study recommends the adoption of the three-digit method of designation (XYZ), developed by the Western Forest Tree Seed Council in 1966 (Buck *et al.*, 1970), as a coding system for seed zones. The first digit indicates the Biogeographic Units (X), the second designates the Region of Provenance (Y), and the third precise the Seed Zone (Z) within the RoP. The value of Z, written in Arabic numerals system, represents the top of the elevation range band where germplasm was collected, as follows: Z=5 for [0-500 m] range band, Z=10 for [501-1000 m] range band, Z=15 for [1001-1500 m] range band, Z=20 for [1501-2000 m] range band, Z=25 for [2001-2500 m] range band, Z=30 for [2501-3000 m] range band, Z=35 for [3001-3500 m] range band, Z=40 for [3501-4000 m] range band and Z=45 for [4001-4500 m] range band.

Within these Seed Zones, genetic material can be safely transferred from a source environment to a planting environment, with preservation of local gene pools and prevention of mal-adapted genotype introduction.

CONCLUSION

To preserve forest genetic resource in Morocco and avoid introducing nonlocal and potentially mal-adapted genotype, it is necessary to identify areas that ensured safe movement of forest germplasm and to express them spatially.

The current paper is a pioneer study that uses accurate geospatial data and long-time-series climate dataset, to provide a detailed bioclimatic and physical characterization of Regions of Provenance.

In this study, Moroccan territory was hierarchically structured into 19 Regions of Provenance concentrated in 9 Biogeographic Units. The environmental characteristics of each RoP were also described with an ensemble of eight environmental variables (6 bioclimatic and 2 physical variables), related to the local adaptation of forest species.

The calculation procedure of the statistical parameters related to various variables for each Region (mean, standard deviation, maximum and minimum values), was performed with zonal GIS techniques, by incorporating polygons of the RoP's vector maps and the raster maps of the involved parameters.

This study is an attempt to provide a precise regionalization scheme to manage forest genetic resources. It is significant that this study enhances the understanding of biogeographical arrangement of the Moroccan territory and investigates physical and bioclimatic homogeneity across each region.

In addition, it shows the interest of inclusion of elevation bands to the proposed Regions of Provenance, in order to improve the intra-region homogeneity.

Therefore, further studies on intra-specific local adaptation by species, should be conducted in order to refine the range of altitudinal bands, by studying other criteria that we have not considered, such as: microclimate (frost corridor, side effect), soil characteristics (acidity, salinity, compaction, mineral wealth, rooting depth, waterlogging), extreme weather events (drought episode, heatwave), biotic risks (fungal pathogen infection, insect pest attack), abiotic risks (forest fire, storms), and human impacts (land cover, silviculture).

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LENGTH-WEIGHT RELATIONSHIP OF FOUR FISH SPECIES FROM RIVER MORAČA, MONTENEGRO

SUMMARY

This study provides data on the length-weight relationships (LWR) of four fish species: *Barbus rebeli* Koller, 1926, *Chondrostoma ohridanum* Karaman, S., 1924, *Pachychilon pictum* (Heckel & Kner, 1858) and *Telestes montenegrinus* (Vuković, 1963) from River Morača, Montenegro. Also, the paper provides the first comprehensive data of LWRs for endemic species for *Telestes montenegrinus* for which no LWR information was available in Fish Base. Values of b parameter ranged from 2.613 to 3.343, while values of a parameter ranged from 0.004-0.038. The results of this study could provide some additional insight into the ecology of the species and useful information for fisheries management in the studied river.

Keywords: Adriatic basin, regression, fisheries, growth, ecology

INTRODUCTION

The autochthonous ichthyofauna of the Adriatic basin of Montenegro consists of 43 species, while 14 of them are non-native species (Marić, 2019). The total length of large rivers and their tributaries in Montenegro is about 1700 km or about 2100 ha of water surface (Burić, 2010). Their special value is their good water quality, so they also represent a rich potential resource for both recreational fishing and aquaculture. The Morača River is the largest river in the Adriatic Basin of Montenegro and largest tributary of Skadar Lake which brings around 62% of water to the Lake (Marić *et al*. 2022). The Morača River is one of the few free-flowing rivers remaining in Europe. It is a biodiversity hotspot: home to several protected fish species such as the endangered endemic species, *Gobio skadarensis* Karaman, S., 1936, found only in Lake Skadar and the lower part of River Morača. The autochthonous ichthyofauna of the River Morača is represented by 24 species (Marić, 2019). Until now, there were no available data

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on LWRs of fish species from river Morača except for the Salmo farioides, Karaman, S., 1937 (Marić and Rakočević, 2015). So far data on the investigate species from the study area was presented as data on presence and distribution (Marić, St. *et al.,* 2010; Marić and Milošević, 2011; Marić, 2019) except for the *T. montenegrinus* which is endemic species for Skadar Lake basin and was subject of some biological and ecological investigation (Drecun *et al.,* 1985; Krivokapić, 1992; Krivokapić, 1998; Krivokapić, 2002, 2002 a; Krivokapić, 2003, 2003 a). Length-weight (L-W) relationship is one of the most widely used methods in fisheries research and its importance has been well documented. A LWRs study for a species can provide important insights into the ecology of the species (Froese, 2006). This parameter can be used to assess the well-being of individuals and is a useful tool in environmental monitoring programs, also (King, 2007).

This paper aimed to report for the first-time length-weight parameters for four species from river Morača, including one endemic for which no estimates were available in Fish Base (Froese and Pauly, 2014).

MATERIAL AND METHODS

The Morača River originates at an elevation of 975 m above sea level (Ljevište), by merging a large number of occasional and permanent streams, which flow from the eastern slopes of mountains Zebalac, Šuplja stijena and the northern slopes of Moračka Kapa Mt. (Drecun *et al*., 1985) (Fig 1). The total length of the Morača River (from the spring to Skadar Lake) is 97 km. The area of the Morača River Basin is 3,257 km^2 (Hrvačević, 2004) and most of it (about 93%) is located in Montenegro. Only the upper part of the Cijevna River Basin is located in Albania. Morača River can be divided into three parts by environmental conditions (physical-chemical environmental parameters): the upper part (from the spring to Medjuriječje), the middle part (from Medjuriječje to Podgorica) and the lower part (from Podgorica to Skadar Lake) (Burić *et al.,* 2010).

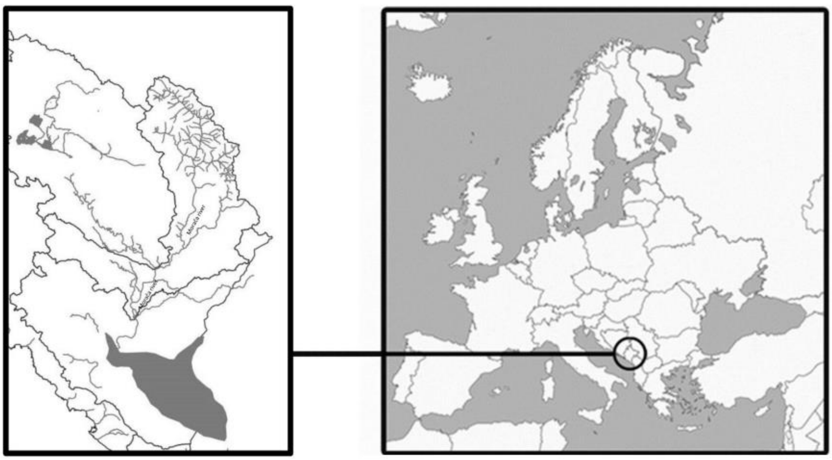

Figure 1. Morača river within the Adriatic basin of Montenegro

Sampling

Research on the Morača River was conducted during the 2019. Fish were sampled by electrofishing and commercial or standard benthic MMG nets (European standard EN 14757 - European Committee for Standardization 2015). A standard electrofishing gear (SUSAN-735MP) was employed according to technical instructions. Species were identified in the field, measured to the nearest 1 mm (total length, TL) and weigh to the nearest 0.1 g (weight, *W*). The mathematical function for estimation of LWRs was (Ricker, 1975): $W=aL^b$, where W is total weight (in g); *L* is total length (TL, in cm); a and b are the coefficients of the functional regression between *W* and *L*. The 95% confidence intervals (Cls) of the parameters and the statistical significance of the regression relationship (r^2) were estimated. The values of function parameters a and b were estimated by linear regression analysis based on the log transformed equation log W=log a+b (log *L*) (Ricker, 1975). The determination coefficient (r^2) was used as an indicator of the quality of the linear regressions.

RESULTS AND DISCUSSION

The sample size, the minimum, maximum, and mean lengths and weights, the values of a and b with their respective 95% confidence limits and the coefficient of determination r^2 for each species are given in Table 1.

Table 1. Descriptive statistics and estimated parameters of LWR for four freshwater fishes from River Morača; *: indicates a difference of b value from 3 (t-test; p<0.005)

		Length (cm)		Weight (gr)		Regression parameters		
Species	N	Min	Max	Min	Max	*b*	*a*	r^2
Barbus rebeli	202	8.7	24.1	12.1	188.1	2.613*	0.038	0.955
Chondrostoma ohridanum	50	14.8	31.1	28.9	257.8	3.025	0.008	0.974
Pachychilon pictum	56	7.5	18.1	3.8	72.3	3.343*	0.004	0.981
Telestes montenegrinus	85	9.1	16.7	9.8	49.6	2.776*	0.019	0.994

The b values ranged from 2.613 for Barbus rebeli to 3.343 for *Pachychilon pictum*. The results of this study are in accordance with Froese (2006), who reported that b values for teleost fish should fall within the expected range of 2.5 and 3.5. For one species, the b values were higher than 3 (t-test; $p<0.05$), for two the b values were lower than 3 (t-test; $p<0.05$), while for *Chondrostoma ohridanum* the b values of the L-W relationships were 3 (Table 1). Values of a parameter ranged from 0.004-0.038. Froese (2006) demonstrate through a meta-analysis that 90% of the intercept values ranged between 0.001 and 0.05. In our study, all species showed a values within the range presented by Froese (2006).

For *C.ohridanum* the results of this study are in concordance with Milošević and Mrdak (2016) who reported b value for *C. ohridanum* from Lake Skadar (b–3.040). For *B. rebeli* and *P. pictum* this data represent the first LWRs data from Montenegro. We found negative allometric growth for *B. rebeli*, (b-2.613). These results differ from the available results of Jordanova *et al*., 2020 who reported almost isometric growth for *B. rebeli* (b-2.994) from Crn Drim (Macedonia). For *P. pictum* we found positive allometric growth (b-3.343). These results are in concordance with the available data for this species from Ohrid Lake and artificial Lake Debar (North Macedonia), respectively (b-3.285; b-3.110) (Milošević and Talevski, 2016). In general, the variations in LWRs of fishes may occur according to sex, gonad maturity, season, habitat type, health, food availability, environmental condition, degree of stomach fullness, differences in the length range of the caught specimens, sampling procedure and fishing gear (Bagenal and Tesch, 1978; Wootton, 1990; Froese, 2006). Also, field measurement can be fluctuating according to differences in fish surface wetness; boat movements and other adverse environmental conditions (Gutreuter and Krzoslen, 1994).

It is noteworthy that for Telestes montenegrinus, which is endemic species for the Skadar Lake basin, this data represents the first description of LWRs based on Fish Base (Froese and Pauly, 2014) Species is Natura 2000 species, also.

CONCLUSIONS

In conclusion, this study provides information on length-weight relationships of four freshwater fish from the Morača River, Montenegro. The length-weight relationship for *Telestes montenegrinus*, which is endemic species for the Skadar Lake basin is provided for the first time. The results of this study could provide data of the relative condition of fish population and fisheries management in the studied river and some additional insight into the conservation of these species.

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ECOLOGY-AND GEOGRAPHY-RELATED FEATURES OF WINTER WHEAT VARIETIES FOR THE AREAS OF INSUFFICIENT HUMIDIFICATION

SUMMARY

The studies on the growth and development characteristics were carried out, as well on the peculiarities of phenophases and photosynthetic activity, yield features, its structure and grain quality of twenty bread winter wheat varieties, of which 11 genotypes represented the entire ecological and geographical diversity of the genetic material for all zones and all leading research institutions of Ukraine, one variety of CIS breeding, 8 varieties bred in various parts of the European Union. The varieties with a stable high yield have been identified, model features that have direct effect on its formation have been shown. The effect of photosynthetic activity on the yielding capacity formation has been revealed, its features have been shown. Grain quality indicators such as protein and gluten content, availability of reserve protein components have been studied. Varieties with high and satisfactory combinations of grain yielding capacity and quality have been identified, promising donors improving these features have been listed, key problems in the current compositions of components of reserve proteins in wheat grain have been described. It is planned to conduct studies in terms of the availability of individual useful micronutrient elements in these varieties.

Keywords: bread winter wheat; yield; yield structure; grain quality; photosynthetic activity; winter resistance.

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INTRODUCTION

Yield and cereal grain quality are the key challenges for the food security in any country in the world. A special place in the diet of the population of Ukraine (as well as of the most Eastern European countries) belongs to winter wheat with world gross yield of 740–780 million tons). However, in the steppe regions characterized by permanent lack of humidity (particularly in the critical phases of crop development) and with high peak temperatures (particularly during the grain ripening season), it is quite difficult to ensure stability in the yield of winter wheat agrocenosis (Daryanto *et al*, 2017).

Thus, the selection of stable and, at the same time, sufficiently plastic genotypes (cells) of this crop becomes not only a priority (Yakymchuk *et al*, 2021), but also a rather non-trivial task that requires continuous studies of both varieties of local (national) breeding and the varieties of world genetic resources (Hongjie *et al*, 2019; Essam *et al*, 2019). The set of samples of varieties of the Dnipro State Agrarian and Economic University, being one of the leading institutions for the subzone of the North of the Ukrainian Steppe (and for the Steppe of Ukraine as a whole), totals 406 constantly updated samples, mainly covering the varieties of the former USSR (from Awnless 1), national breeding varieties, modern varieties bred in the CIS countries and Western Europe. A particular focus is set on comparing the successes of national breeding and breeding of leading scientific institutions in Western Europe, both in terms of adaptability to local conditions and comparison in terms of yielding capacity and quality indicators (Bordes *et al*, 2011; Bondarenko and Nazarenko, 2020; Mangi *et al*, 2021).

Key attention is paid annually to the grain yielding capacity, its structure (identification of key components that provide superiority in yield), grain quality parameters (protein content, gluten content, individual units of high molecular weight glutenins and the availability of valuable components of gliadins) (Tokatlidis, 2017; Li *et al*, 2019). In addition, the study of drought resistance (using both visual assessment and laboratory methods), and winter resistance (the same way) is carried out, although in a more limited mode (Bordes *et al*, 2011; Lykhovyd, 2021).

The complex of a thorough and comprehensive assessment of winter wheat genotypes was commonly named an environmental test (Xu, 2016; Tengcong *et al*, 2020). Considering rather active climate changes (global warming) (Le Gouis *et al*, 2020; Avtaeva *et al*, 2021b) it should be noted that in general, for such semi-arid regions as the Steppe of Ukraine it has led to mostly positive consequences which were expressed in relaxing the wintering conditions, in the increase of not only the total amount of precipitation, but also the rates of precipitation during periods of heading and grain formation, which are critical for winter wheat. In general, the recorded changes are rather positive for winter crops (Liu *et al*, 2017; Horshchar and Nazarenko, 2022), but they also require correction to create the right energy balance in the variety model. Moreover, the problem of improving the quality of grain requires additional attention (Vesali *et* *al*, 2017). Although it is primarily related to the issues of compliance with the cultivation technology, it still remains incompletely solved from the point of view of the genetic component (variety) (Kozak *et al*, 2020). In this part of the study, a group of contrasting varieties (under the conditions of the Northern Steppe of Ukraine, i.e. the semi-arid zone) of different origin (20 varieties in total) has been assessed in terms of yielding capacity, grain quality and ecological plasticity.

MATERIAL AND METHODS

Field experiments have been carried out on the research field of the Dnipro State Agrarian and Economic University in 2021-2023. In total, 20 winter wheat (*Triticum aestivum* L.) varieties were tested, the area of the experimental plots was 5m^2, three replications, the variety Podolyanka was used as a reference, being the most stable variety in terms of characteristics presenting in the conditions of Ukraine. The following varieties were sown: Podolyanka, Samara 2, Perspektyva Odeska, Perlyna Podillia, Sonata Poltavska, Shpalivka, Zoreslav, Grom, Zoryanka, Poradnytsia, MIP Lada, Syla, Farell, Amandus, NE 12443, Retezat, Ronin, Patras, Seilor, Azano.

Weather conditions for hydrothermal indicators in the years of research (2021–2023) varied, which made possible to obtain objective results, for the location of the research fields: air temperature during winter wheat growing season 2022 (September–July) was 9.0°C, the average rainfall is about 611.7 mm; air temperature in season 2022 was 9.7°C, the average rainfall is about 573.4 mm; air temperature in season 2023 was 8.9°C, the average rainfall is about 562.0 mm). The following activities were carried out: phenological observations during the vegetation of winter wheat varieties, assessment of wintering, assessment of photosynthetic activity, assessment of yielding capacity, analysis of elements of the yield structure, analysis of grain quality (percentage of gluten and protein, availability of gliadins and glutenins).

Winter resistance was evaluated by the concentration of soluble sugars, determined at the tillering nods of varieties according to generally accepted GOST 26176-91. Yield structure was determined by standard parameters in triplicate, the sample was 25–30 plants including the marginal effects (plant height, parameters of the main ear, plant yield, thousand grains weight (TGW). There has been conducted the phlenological studies, assessed the overwintering of both visually and by determining the concentration of sugars in the node in meaningful period, determined the germination and survival of plants on the plots, conducted an assessment of photosynthetic activity during the earing period by the SPAD-502 appliance and an calculation to the concentration of chlorophyll (a+b) according to the generally accepted methodology by the formula Chl=10M^0.265, where M is the value of SPAD units (Vesali *et al*, 2017). Agrochemical analysis of soils for content of nutrient elements was provided too (N-NO3, mg kg-1 18.7–32.8, P2O5 14.8–27.1, K2O 134–235). The protein content and contents of gliadin and glutenin were identified on device Spectran RT (for protein content) and RP-HPLS (for gliadins and glutenins) (Bordes *et al*, 2011).

Statistical analysis of the results was conducted in Statistica 10.0. (TIBCO, Palo Alto, USA). Values in the tables are given as x±SD (mean ± standard deviation). The differences between the selections were determined using single-factor dispersion analysis (ANOVA) and were considered reliable at $P<0.05$. The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSION

The studied varieties were selected in such a way as to, on the one hand, reflect the biodiversity used in domestic breeding to the fullest, and on the other hand, to provide enough data for comparison on the varieties bred in the countries, the resources of which are actively used in the sub-zone of the Northern Steppe of Ukraine in the maximum diversity of phenology (Table 1). A total of 20 genotypes have been presented – Podolyanka as a reference, being the most stable genotype in terms of growing in the widest range of conditions, and varieties Samara 2, Perspektyva Odeska, Perlyna Podillia, Sonata Poltavska, Shpalivka, Zoreslav, Grom, Zoryanka, Poradnytsia, MIP Lada (bred in Ukraine, various centres from Polissia to the South of Steppe), Syla (Russian Federation), Farell, Amandus, Retezat, Ronin, Patras, Seilor, Azano (EU breeding), NE 1244 (CYMMIT).

Table 1. General characteristic of winter wheat varieties phenotype

Variety	CoG	Awns	PH	Maturing	Type	Days
Podolyanka	Ukr	awnless	m	m	semiintensive	272
Samara 2	Ukr	awn	m	e-m	intensive	269
Perspektyva Odeska	Ukr	awn	m	m	intensive	271
Perlyna Podillia	Ukr	awnless	m	m	semiintensive	273
Sonata Poltavska	Ukr	awn	m	m	intensive	272
Shpalivka	Ukr	awn	s	m	intensive	274
Zoreslav	Ukr	awn	s	m	intensive	274
Grom	Ukr	awnless	s	l	intensive	280
Zoryanka	Ukr	awnless	m	m	intensive	271
Poradnytsia	Ukr	awn	m	e-m	intensive	269
MIP Lada	Ukr	awnless	h	m	semiintensive	274
Syla	Rus	awnless	sd	m-l	intensive	276
Farell	Ca	awnless	m	l	intensive	281
Amandus	Au	awn	m	m	intensive	274
NE 12443	Me	awn	h	m	semiintensive	273
Retezat	Ro	awn	s	l	intensive	281
Ronin	Deu	awnless	s	m-l	intensive	275
Patras	Deu	awnless	sd	l	intensive	283
Seilor	Fr	awn	m	l	intensive	284
Azano	Swe	awnless	sd	l	intensive	286

Note: CoG – country of origing; Ukr – Ukraine; Rus – Russian Federation; Ca – Canada; Me – Mexica; Ro – Romania; Swe – Sweden; Fra – France; Deu – Deutschland; PH – plant height; h – high-stem; m – medium; s – short-stem; sd – semidwarf; e-m – early-medium; m-l – medium-late; l – late.

Among the varieties, both awnless (potentially more resistant to entopests) and awned forms are equally represented, and it can be noted that there is no focus here. Mostly varieties of domestic breeding are medium-grown, although there are three short-stalked varieties. The varieties of foreign breeding are mainly of a more intensive type, there is a significant number of semidwarfs, which cannot be found among domestic varieties. As for the maturity period (according to the data regarding the heading stage), the domestic varieties are predominantly midseason maturing and early maturing, while foreign varieties tend to be late maturing, which on the one hand allows to better fulfil the yield potential, however, does not help to avoid droughts during the critical period of grain formation in our region from mid-May to early June. However, due to climate change, the situation has slightly changed in the last five years. No early maturing varieties have been observed, which makes different combinations of geno-types slightly more vulnerable (it is still advisable to use at least 10% of early maturing forms when sowing).

The varieties are mainly referred to intensive forms (in terms of the tuft form, the peculiarities of growth and development, the requirements for technology), but among the domestic forms, almost a third are semiintensive. Among foreigners, only the collection sample is considered intensive, which is mainly used as a source material and a reference form due to its properties. The number of days to full maturity and harvesting shows that the EU breeding is still focused on forms with a longer growing season, and it is sufficient to record the date of spiking in order to identify the peculiarities of development, and there has not been done anything particular in terms of the duration of individual phases (attempts to create the forms, would be photosynthesizing for a longer period after ear formation, which would allow more effective use of the plant's potential with the outflow of nutrients for grain formation).

One of the key parameters for the region is winter resistance (Table 2), which has been studied both through visual evaluation and measuring of sugars in the tillering node depending on the period (three measurements). In general, the visual assessment correlates quite well with the obtained laboratory data, the trait was conditioned both by the genotype of the variety ($F=9.27$; $F_{0.05}=6.01$; $P=3.8*10^{-7}$) and by the year of cultivation ($F=11.46$; $F_{0.05}=3.88$; $P<0.01$). The general tendency to develop winter resistance allows us to say that the more successful forms in this regard include varieties of domestic breeding and the Russian variety ($F=24.92$; $F_{0.05}=4.12$; $P=2.1*10^{-16}$), while foreign varieties are generally worse in terms of the concentration of sugars and outcomes of visual assessment. The following genotypes were distinguished by winter resistance: Podolyanka ($F=26.44$; $F_{0.05}=4.12$; $P=1.2*10^{-14}$), Sonata Poltavska ($F=18.16$; $F_{0.05}=4.12$; $P=5.1*10^{-12}$), MIP Lada ($F=27.49$; $F_{0.05}=4.12$; $P=1.9*10^{-13}$), Syla ($F=31.22$; $F_{0.05}=4.12$; $P=1.7*10^{-15}$) and only one foreign variety Azano ($F=17.01$; $F_{0.05}=4.12$; $P=4.0*10^{-9}$), apparently as a representative of the Scandinavian breeding, where this feature is much more important than for the rest of the EU.

It is also worth noting that worse ability to accumulate the necessary sugars at the first stage of preparing for wintering is not an indication of worse winter resistance. The situation may also change due to the lower consumption for life support, like in the Sonata Poltavska variety, which is obviously more interesting for further research in this area.

Table 2. Winter wheat varieties parameters during winter period (2021/2022 periods of vegetation dates) (x±SD, n=5).

Variety	G	BW	Content of sugars in tillering nod, %			AW
			11	02	03	
Podolyanka	5.0	5.0	34.0 ± 0.3^{a}	26.9 ± 0.4^{a}	21.7 ± 0.3^{a}	5.0
Samara 2	5.0	5.0	32.9 ± 0.3^{b}	25.8 ± 0.3^{b}	21.5 ± 0.4^{a}	5.0
Perspektyva Odeska	5.0	5.0	33.0 ± 0.3^{b}	26.0 ± 0.4^{b}	21.4 ± 0.3^{a}	5.0
Perlyna Podillia	4.8	4.8	31.9 ± 0.4^{c}	25.0 ± 0.4^{c}	20.7 ± 0.3^{b}	4.5
Sonata Poltavska	5.0	5.0	33.1 ± 0.3^{b}	26.2 ± 0.4^{ab}	21.8 ± 0.3^{a}	4.8
Shpalivka	4.8	4.8	31.7 ± 0.4^{c}	24.9 ± 0.4^{c}	20.6 ± 0.3^{b}	4.5
Zoreslav	4.8	4.8	31.8 ± 0.3^{c}	24.8 ± 0.4^{c}	20.5 ± 0.4^{b}	4.3
Grom	4.8	4.8	31.9 ± 0.3^{c}	25.0 ± 0.3^{c}	20.8 ± 0.4^{b}	4.3
Zoryanka	4.8	4.8	31.5 ± 0.5^{c}	24.7 ± 0.4^{c}	20.5 ± 0.4^{b}	4.5
Poradnytsia	4.8	4.8	31.6 ± 0.5^{c}	24.8 ± 0.4^{c}	20.6 ± 0.3^{b}	4.3
MIP Lada	5.0	5.0	34.2 ± 0.3^{a}	27.1 ± 0.4^{a}	21.8 ± 0.4^{a}	5.0
Syla	5.0	5.0	34.4 ± 0.4^{a}	27.2 ± 0.3^{a}	22.2 ± 0.4^{a}	5.0
Farell	4.8	4.8	33.0 ± 0.4^{b}	26.0 ± 0.4^{b}	21.0 ± 0.3^{b}	4.8
Amandus	4.3	4.3	29.5 ± 0.4^{d}	23.8 ± 0.5^{d}	19.0 ± 0.4^{c}	4.0
NE 12443	4.0	4.0	25.1 ± 0.5^{e}	21.4 ± 0.5^{e}	18.0 ± 0.3^{d}	3.5
Retezat	4.3	4.3	29.6 ± 0.4^{d}	23.9 ± 0.4^{d}	19.1 ± 0.4^{c}	4.0
Ronin	4.3	4.3	29.0 ± 0.5^{d}	23.3 ± 0.5^{d}	19.4 ± 0.4^{c}	4.0
Patras	4.3	4.3	29.1 ± 0.5^{d}	23.7 ± 0.4^{d}	19.3 ± 0.4^{c}	4.0
Seilor	4.3	4.3	28.2 ± 0.6^{d}	23.5 ± 0.5^{d}	19.2 ± 0.4^{c}	4.0
Azano	5.0	5.0	33.9 ± 0.3^{a}	27.0 ± 0.5^{a}	21.6 ± 0.4^{a}	5.0

Note: G – germination, BW – evaluation before winter period [balls]; AW – evaluation after winter period [balls]; significant differences at P < 0.05 by factor analyze.

It can be said that for the first time we observe some variability of domestic material in terms of the mechanism of formation of the complex trait. Variety NE 12443 was distinguished for its low winter resistance, which in this case was used as an additional reference point in terms of the lower rates ($F=13.99$; $F_{0.05}=4.12$; $P=6.7*10^{-10}$). In general, varieties Podolyanka, Sonata Poltavska, MIP Lada, Syla and Azano are advantageous for growing in the region taking into account the more severe wintering conditions. However, the increasing tendency to ease winter conditions should be taken into account. The final check of the material using the Tukey's test confirmed all the conclusions made.

The yielding capacity of this set of varieties was being studied for three years (with a more favourable year in 2021) (Table 3), and the grain share in the total biological productivity of wheat was also taken into account. This number depends most on the characteristics of the plant architecture and increases significantly for shorter and more intensive forms, which is demonstrated by a higher value of this feature in shorter varieties of foreign breeding. Variety Patras is particularly distinguished in this regard, but this in itself does not provide any increase in yields.

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Podolyanka	5.0	5.0	34.0 ± 0.3[a]	26.9 ± 0.4[a]	21.7 ± 0.3[a]	5.0
Samara 2	5.0	5.0	32.9 ± 0.3[b]	25.8 ± 0.3[b]	21.5 ± 0.4[a]	5.0
Perspektyva Odeska	5.0	5.0	33.0 ± 0.3[b]	26.0 ± 0.4[b]	21.4 ± 0.3[a]	5.0
Perlyna Podillia	4.8	4.8	31.9 ± 0.4[c]	25.0 ± 0.4[c]	20.7 ± 0.3[b]	4.5
Sonata Poltavska	5.0	5.0	33.1 ± 0.3[b]	26.2 ± 0.4[ab]	21.8 ± 0.3[a]	4.8
Shpalivka	4.8	4.8	31.7 ± 0.4[c]	24.9 ± 0.4[c]	20.6 ± 0.3[b]	4.5
Zoreslav	4.8	4.8	31.8 ± 0.3[c]	24.8 ± 0.4[c]	20.5 ± 0.4[b]	4.3
Grom	4.8	4.8	31.9 ± 0.3[c]	25.0 ± 0.3[c]	20.8 ± 0.4[b]	4.3
Zoryanka	4.8	4.8	31.5 ± 0.5[c]	24.7 ± 0.4[c]	20.5 ± 0.4[b]	4.5
Poradnytsia	4.8	4.8	31.6 ± 0.5[c]	24.8 ± 0.4[c]	20.6 ± 0.3[b]	4.3
MIP Lada	5.0	5.0	34.2 ± 0.3[a]	27.1 ± 0.4[a]	21.8 ± 0.4[a]	5.0
Syla	5.0	5.0	34.4 ± 0.4[a]	27.2 ± 0.3[a]	22.2 ± 0.4[a]	5.0
Farell	4.8	4.8	33.0 ± 0.4[b]	26.0 ± 0.4[b]	21.0 ± 0.3[b]	4.8
Amandus	4.3	4.3	29.5 ± 0.4[d]	23.8 ± 0.5[d]	19.0 ± 0.4[c]	4.0
NE 12443	4.0	4.0	25.1 ± 0.5[e]	21.4 ± 0.5[e]	18.0 ± 0.3[d]	3.5
Retezat	4.3	4.3	29.6 ± 0.4[d]	23.9 ± 0.4[d]	19.1 ± 0.4[c]	4.0
Ronin	4.3	4.3	29.0 ± 0.5[d]	23.3 ± 0.5[d]	19.4 ± 0.4[c]	4.0
Patras	4.3	4.3	29.1 ± 0.5[d]	23.7 ± 0.4[d]	19.3 ± 0.4[c]	4.0
Seilor	4.3	4.3	28.2 ± 0.6[d]	23.5 ± 0.5[d]	19.2 ± 0.4[c]	4.0
Azano	5.0	5.0	33.9 ± 0.3[a]	27.0 ± 0.5[a]	21.6 ± 0.4[a]	5.0

Note: G – germination, BW – evaluation before winter period [balls]; AW – evaluation after winter period [balls]; significant differences at P < 0.05 by factor analyze.

It can be said that for the first time we observe some variability of domestic material in terms of the mechanism of formation of the complex trait. Variety NE 12443 was distinguished for its low winter resistance, which in this case was used as an additional reference point in terms of the lower rates ($F=13.99$; $F_{0.05}=4.12$; $P=6.7*10^{-10}$). In general, varieties Podolyanka, Sonata Poltavska, MIP Lada, Syla and Azano are advantageous for growing in the region taking into account the more severe wintering conditions. However, the increasing tendency to ease winter conditions should be taken into account. The final check of the material using the Tukey's test confirmed all the conclusions made.

The yielding capacity of this set of varieties was being studied for three years (with a more favourable year in 2021) (Table 3), and the grain share in the total biological productivity of wheat was also taken into account. This number depends most on the characteristics of the plant architecture and increases significantly for shorter and more intensive forms, which is demonstrated by a higher value of this feature in shorter varieties of foreign breeding. Variety Patras is particularly distinguished in this regard, but this in itself does not provide any increase in yields.

Table 3. Grain productivity of winter wheat genotypes (2021-2023 years).

Variety	Percent of grains in total productivity, %	Yield, t ha^{-1}			Average
		2021	2022	2023	
Podolyanka	41.20 ±1.12^{a}	7.64 ± 0.11^{a}	6.82 ± 0.09^{a}	6.49 ± 0.18^{a}	6.98 ± 0.21^{a}
Samara 2	40.80 ± 1.22^{a}	7.45 ± 0.23^{a}	6.63 ± 0.19^{a}	6.22 ± 0.19^{a}	6.77 ± 0.23^{a}
Perspektyva Odeska	42.40 ± 1.17^{a}	6.65 ± 0.19^{b}	6.23 ± 0.07^{b}	6.01 ± 0.13^{b}	6.30 ± 0.33^{b}
Perlyna Podillia	40.10 ± 1.19^{a}	6.53 ± 0.15^{b}	6.11 ± 0.17^{b}	5.56 ± 0.10^{c}	6.07 ± 0.29^{b}
Sonata Poltavska	44.20 ± 1.22^{b}	8.12 ± 0.16^{c}	7.42 ± 0.23^{c}	7.26 ± 0.11^{d}	7.60 ± 0.26^{c}
Shpalivka	43.20 ± 1.23^{ab}	7.51 ± 0.21^{a}	6.73 ± 0.20^{a}	6.55 ± 010^{a}	6.93 ± 0.21^{a}
Zoreslav	45.10 ± 1.32^{bc}	7.65 ± 0.19^{a}	6.23 ± 0.15^{b}	7.25 ± 0.22^{d}	7.04 ± 0.23^{a}
Grom	44.70 ± 1.19^{b}	8.71 ± 0.17^{c}	6.84 ± 0.17^{a}	7.65 ± 0.10^{d}	7.73 ± 0.24^{c}
Zoryanka	42.00 ± 1.34^{a}	7.01 ± 0.23^{b}	7.17 ± 0.10^{a}	6.12 ± 0.19^{b}	6.77 ± 0.27^{a}
Poradnytsia	41.00 ± 1.43^{a}	6.95 ± 0.21^{b}	6.77 ± 0.18^{a}	6.00 ± 0.17^{c}	6.57 ± 0.20^{a}
MIP Lada	40.50 ± 1.23^{a}	7.52 ± 0.14^{a}	7.01 ± 0.12^{a}	6.52 ± 0.18^{a}	7.02 ± 0.20^{a}
Syla	48.10 ± 1.39^{d}	8.45 ± 0.14^{c}	7.39 ± 0.21^{c}	7.46 ± 0.22^{d}	7.77 ± 0.29^{c}
Farell	48.70 ± 1.32^{d}	8.01 ± 0.11^{c}	7.17 ± 0.20^{c}	7.42 ± 0.16^{d}	7.53 ± 0.19^{c}
Amandus	49.00 ± 0.98^{d}	6.96 ± 0.09^{b}	6.44 ± 0.12^{b}	6.37 ± 0.11^{a}	6.59 ± 0.32^{a}
NE 12443	40.10 ± 1.53^{a}	6.01 ± 0.15^{d}	5.09 ± 0.22^{d}	5.07 ± 0.16^{e}	5.39 ± 0.24^{b}
Retezat	49.60 ± 1.17^{d}	8.16 ± 0.11^{c}	7.09 ± 0.13^{a}	7.17 ± 0.14^{d}	7.47 ± 0.30^{a}
Ronin	48.50 ± 1.34^{d}	7.99 ± 0.14^{c}	7.81 ± 0.21^{c}	6.67 ± 0.13^{a}	7.49 ± 0.22^{c}
Patras	52.10 ± 0.75^{e}	6.92 ± 0.22^{b}	6.47 ± 0.14^{b}	6.32 ± 0.19^{ab}	6.57 ± 0.31^{a}
Seilor	47.90 ± 1.12^{d}	7.98 ± 0.19^{a}	7.19 ± 0.21^{c}	6.94 ± 0.15^{d}	7.37 ± 0.24^{c}
Azano	48.90 ± 1.49^{d}	7.92 ± 0.20^{a}	7.35 ± 0.23^{c}	7.32 ± 0.19^{d}	7.53 ± 0.24^{c}

Note: significant differences at P < 0.05 by factor analyze

The yields characteristics depended both on the genotype of the variety ($F=7.11$; $F_{0.05}=6.01$; $P=0.02$) and on the year of cultivation ($F=14.62$; $F_{0.05}=3.88$; $P=1.4*10^{-11}$).When we analysed the characteristics by individual varieties, we found that the following genotypes were positively distinguished by this feature: Sonata Poltavska ($F=11.16$; $F_{0.05}=3.55$; $P=3.2*10^{-10}$), Grom ($F=9.07$; $F_{0.05}=3.55$; $P=4.7*10^{-6}$), Syla ($F=13.17$; $F_{0.05}=3.55$; $P=9.0*10^{-9}$), Farell ($F=9.08$; $F_{0.05}=3.55$; $P=5.1*10^{-9}$), Ronin ($F=10.33$; $F_{0.05}=3.55$; $P=1.7*10^{-8}$), Seilor ($F=6.19$; $F_{0.05}=3.55$; $P=0.03$), Azano ($F=11.49$; $F_{0.05}=3.55$; $P=2.6*10^{-10}$), which, according to the results of three years of testing, exceled the Podolyanka variety as a yield standard for the region. However, two of the last-named varieties were at the reference variety level in the most favourable year 2020, which was confirmed during the Tukey's test. For a more accurate classification of varieties depending on variability by years, a cluster analysis was carried out (Fig.1), which allowed distinguishing 7 groups of varieties by yielding capacity depending on variability by years and genotypes, among which there were 4 minor ones (represented by one variety).

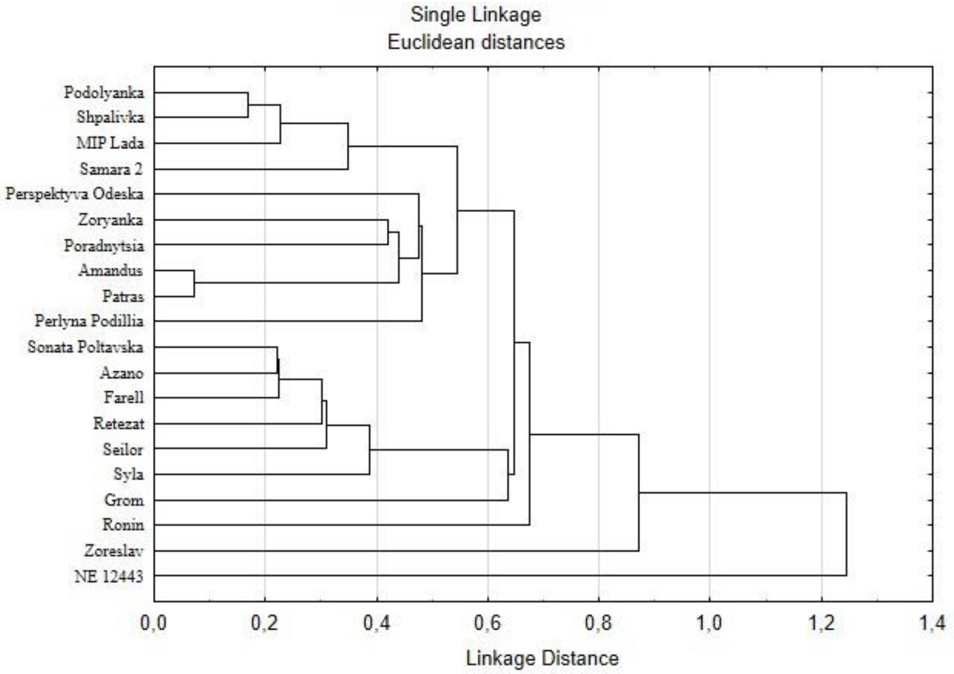

Figure 1. Resaults of cluster analysis by grain productivity.

Shpalivka, and MIP Lada genotypes. A high stability of this feature is expected with a significant mitigation of the impact of the conditions during the year of growing (high ecological plasticity). The second group is represented by varieties Perspektyva Odeska, Zoryanka, Poradnytsia, Patras, Amandus, and Perlyna Podillia. In general, these varieties are significantly inferior to the standard, but in some years, they can show yields close to it. The third group includes Sonata Poltavska, Azano, Syla, Farell, Retezat, Seilor – the varieties are significantly superior to the standard in all years of testing without variants. These varieties are most promising for the Northern Steppe of Ukraine. The fourth minor group includes the Grom variety, which is generally superior to the reference variety. However, it did not differ statistically significantly from the reference in the most favourable year. The variety is quite promising in terms of yielding capacity, but it is not very stable. The fifth group, which includes variety Ronin showed lower yields, but in the conditions of an unfavourable year was at the level of the standard variety. The sixth group, variety Zoreslav, is unstable in terms of growing conditions, it can both excel the standard or be at the same level, and be inferior to it, but on average it is at the level of the standard variety. The seventh group, variety NE 12443, is significantly inferior to both the standard and the least yielding group, without variations. In general, the varieties of the third and fourth minor groups are definitely better, based on the yielding capacity figures.

In order to determine the mechanism of yield formation, a structural analysis was carried out (Table 4) in terms of the parameters as follows: plant height, number and weight of grain in the main ear, weight of grain of the plant, thousand grain weight (hereinafter referred to as TGW). When it comes to the plant height, the structural analysis confirmed previous visual characteristic of wheat varieties. In general, foreign varieties are characterized by short height or semi-dwarfism with an advantage in terms of the plant architecture of a long grained spike. It can also form additional full ears with a high nitrogen content. The main ear grain quantity indicator is extremely variable and it can perhaps be noted that not only high-yielding, but also low-yielding varieties can have a significant advantage of this parameter over the reference ones, and only a high combination of this feature with the grain quality can be of significance, which the second characteristics show – the weight of grain in the main ear, by which such varieties as Sonata Poltavska, Farell, Ronin, and partially varieties Seilor and Azano were significantly distinguished – that is, only those varieties that significantly excelled in terms of productivity as well ($F=8.17$; $F_{0.05}=6.01$; $P=0.03$). Obviously, for these varieties, the formation of yield as an integrative feature (in terms of structure) is precisely due to the well-grained main ear of high quality. Also, for the variety NE 12443 ($F=12.64$; $F_{0.05}=3.55$; $P=4.1*10^{-14}$), the low level of this feature has also become a significant problem of low yielding capacity.

Table 4. Parameters of main components of yield structure (at average) (x±SD, n=25).

Variety	PH	Per main spike		WGP	TGW
		GN	GW		
Podolyanka	101.0 ± 1.3^{a}	35.5 ± 3.7^{a}	1.7 ± 0.2^{a}	4.0 ± 0.4^{a}	50.0 ± 3.2^{a}
Samara 2	99.8 ± 1.7^{a}	34.0 ± 4.8^{a}	1.5 ± 0.3^{a}	4.4 ± 0.4^{a}	45.5 ± 2.8^{a}
Perspektyva Odeska	92.2 ± 1.7^{b}	32.8 ± 2.9^{a}	1.4 ± 0.2^{a}	3.2 ± 0.3^{b}	42.7 ± 2.2^{ab}
Perlyna Podillia	89.0 ± 2.3^{b}	36.6 ± 3.0^{a}	1.4 ± 0.1^{a}	3.8 ± 0.3^{a}	42.0 ± 2.1^{b}
Sonata Poltavska	93.4 ± 1.9^{b}	41.2 ± 7.4^{b}	2.2 ± 0.2^{b}	4.9 ± 0.3^{c}	55.5 ± 2.3^{d}
Shpalivka	99.2 ± 1.5^{a}	37.5 ± 5.4^{a}	1.6 ± 0.2^{a}	4.2 ± 0.2^{a}	46.5 ± 2.1^{a}
Zoreslav	77.0 ± 1.4^{c}	47.8 ± 2.7^{b}	1.7 ± 0.2^{a}	4.7 ± 0.4^{a}	45.6 ± 2.3^{a}
Grom	76.0 ± 1.6^{c}	47.2 ± 3.7^{b}	1.9 ± 0.3^{a}	5.1 ± 0.3^{c}	56.2 ± 2.0^{d}
Zoryanka	95.4 ± 2.8^{a}	48.3 ± 4.0^{b}	1.4 ± 0.2^{a}	3.8 ± 0.3^{a}	39.3 ± 2.3^{b}
Poradnytsia	97.3 ± 1.2^{a}	45.0 ± 9.7^{b}	1.3 ± 0.2^{a}	4.5 ± 0.3^{a}	44.7 ± 2.0^{a}
MIP Lada	112.3 ± 2.9^{d}	39.6 ± 5.5^{a}	1.5 ± 0.2^{a}	3.9 ± 0.2^{a}	46.9 ± 2.1^{a}
Syla	57.6 ± 2.4^{e}	43.3 ± 4.1^{b}	2.1 ± 0.2^{a}	5.2 ± 0.3^{c}	55.9 ± 2.5^{d}
Farell	93.1 ± 1.4^{b}	32.3 ± 3.9^{a}	2.2 ± 0.1^{b}	5.0 ± 02^{c}	55.6 ± 2.1^{d}
Amandus	89.2 ± 1.7^{b}	43.3 ± 5.6^{b}	1.8 ± 0.2^{b}	3.5 ± 0.3^{b}	39.5 ± 1.9^{b}
NE 12443	112.4 ± 2.3^{d}	41.3 ± 4.6^{b}	1.2 ± 0.2^{c}	3.4 ± 0.3^{b}	38.2 ± 2.9^{bc}
Retezat	78.5 ± 1.4^{c}	39.3 ± 4.1^{a}	1.8 ± 0.1^{a}	4.1 ± 0.3^{a}	48.5 ± 2.1^{a}
Ronin	76.5 ± 1.1^{c}	46.1 ± 3.9^{b}	2.2 ± 0.2^{b}	4.7 ± 0.3^{ac}	51.5 ± 1.9^{ae}
Patras	77.0 ± 1.4^{c}	40.4 ± 4.2^{a}	1.8 ± 0.1^{a}	4.0 ± 0.3^{a}	40.0 ± 2.5^{b}
Seilor	88.6 ± 1.3^{b}	48.2 ± 5.1^{b}	1.9 ± 0.2^{ab}	4.9 ± 0.5^{ac}	55.7 ± 2.0^{d}
Azano	58.3 ± 1.7^{e}	42.3 ± 4.3^{b}	1.9 ± 0.2^{ab}	4.6 ± 0.3^{a}	56.5 ± 1.8^{d}

Note: PH – plant height[cm]; GN – grain number[piece]; GW – grain weight[g]; TGW – thousand grain weight [g]; WGP – weight of grain per plant [g], significant differences at $P < 0.05$ by factor analyze.

The following indicator of grain weight per plant has already become significant for advantageous yield for Sonata Poltavska, Grom, Syla, Farell, Azano, partially Ronin and Seilor ($F=14.83$; $F_{0.05}=4.88$; $P=1.6*10^{-12}$), which allows us to conclude that for Grom and Syla varieties, the formation of a greater number of well-grained ears is of greater importance than that of the main ears, and for Seilor and Azano varieties a mixed option is possible, when both the main ear and additional ears are of great importance, which offers numerous opportunities of combining the elements of growing technology. The following TGW indicator expressly exceeded the standard for all high-yielding varieties: Sonata Poltavska, Grom, Syla, Farell, Azano, Ronin and Seilor, which indicates the key role of grain quality in yield formation. Thus, in varieties Sonata Poltavska, Farell, and Ronin, the formation of high yield depends on the main ear, the Grom and Syla varieties have high-performance tilling capacity, while in Seilor and Azano varieties have a mixed mechanism.

A study of photosynthetic activity (Table 5), in turn, showed that the higher yielding varieties were also significantly superior in terms of this parameter in the heading stage ($F=11.483$; $F_{0.05}=6.01$; $P=1.2*10^{-11}$). Only one more variety Shpalivka was added, which had a yielding capacity at the reference level. This gives us the opportunity to talk about the relationship between yielding capacity and high photosynthetic activity during the heading stage. However, less productive varieties do not necessarily have lower photosynthetic activity. The same results were obtained by pairwise comparison using Tukey's test.

Table 5. Parameters of photosynthetic activity. First group (x±SD, n=5).

Variant	Soil Plant Analysis Development (SPAD)	Chl, $\mu mol/m^2$
Podolyanka	50.39 ± 1.45[a]	669.37 ± 12.69
Samara 2	49.11 ± 1.63[a]	640.42 ± 13.75
Perspektyva Odeska	49.50 ± 1.36[a]	649.17 ± 13.34
Perlyna Podillia	45.07 ± 1.51[b]	553.79 ± 13.04
Sonata Poltavska	55.11 ± 0.60[c]	782.56 ± 7.47
Shpalivka	55.98 ± 0.68[c]	804.55 ± 8.00
Zoreslav	50.11 ± 0.64[ac]	662.98 ± 7.73
Grom	58.12 ± 0.46[c]	860.17 ± 6.52
Zoryanka	49.18 ± 0.39[a]	641.99 ± 6.01
Poradnytsia	47.16 ± 1.20[b]	597.72 ± 11.21
MIP Lada	50.94 ± 1.44[b]	681.34 ± 12.63
Syla	52.44 ± 0.89[c]	717.28 ± 9.32
Farell	54.91 ± 0.99[c]	777.55 ± 9.94
Amandus	49.74 ± 1.15[a]	654.58 ± 10.91
NE 12443	48.14 ± 1.42[a]	618.97 ± 12.51
Retezat	51.40 ± 1.12[a]	692.74 ± 10.73
Ronin	55.17 ± 1.13[a]	784.06 ± 10.79
Patras	49.42 ± 2.45[a]	647.37 ± 18.54
Seilor	53.49 ± 1.00[c]	742.56 ± 10.00
Azano	54.12 ± 1.12[c]	757.97 ± 10.73

Note: significant differences at P < 0.05 by factor analyse

To measure the weight of each trait and its model value, a factorial and discriminant analysis were carried out, respectively, in order to identify both key traits affecting the formation of grain productivity and the possibilities for their classification for each genotype (Table 6, 7). As a result, it was found that the most frequent models were the parameters of sugar concentration at the beginning and at the end of winter, grain weight per plant, TGW, and photosynthetic activity. Essentially more parameters were valid for the genotype (variety). In this case, such parameters as the height of the plants and the weight of grains of the main ear were important.

Table 6. Factor Loadings (Unrotated) and Discriminant Function

Parameter	Year	Genotype	Wilks' - Lambda	F_{remove} (6.12)	p-value
CS 11	-0.848*	0.892*	0.018	9.64	< 0.01
CS 02	-0.654	0.404	0.011	4.11	0.09
CS 03	0.748*	0.861*	0.018	8.95	0.04
PH	0.532	0.792*	0.017	8.16	0.04
GN	0.311	0.317	0.010	3.23	0.12
GW	-0.611	0.788*	0.017	7.98	0.10
WGP	0.812*	0.912*	0.021	14.12	< 0.01
TGW	0.748*	0.943*	0.028	18.92	< 0.01
SPAD	0.850*	-0.893*	0.020	11.43	< 0.01
Explenation variants	2.162	1.976	–	–	–
Non-explanation	0.893	0.198	–	–	–

Table 7. Resaults of classification for genotypes (part of objects by parameters from previous table in model for such genotype)

Genotype	Objects in model, %
Podolyanka	100.0
Samara 2	100.0
Perspektyva Odeska	71.4
Perlyna Podillia	57.1
Sonata Poltavska	85.7
Shpalivka	71.4
Zoreslav	85.7
Grom	100.0
Zoryanka	71.4
Poradnytsia	57.1
MIP Lada	85.7
Syla	71.4
Farell	85.7
Amandus	71.4
NE 12443	57.1
Retezat	71.4
Ronin	100.0
Patras	71.4
Seilor	85.7
Azano	100.0

In turn, the results of discriminant analysis clearly show that the concentration of sugars at the beginning and at the end of winter, the weight of grain of the main ear and per plant, TGW, and photosynthetic activity are of primary importance for modelling the future yield in terms of varietal response for specific environmental conditions. The successful classification of individual genotypes shows that at least four parameters from the set are always significant, although the set itself varies significantly depending on the specific genotype. At the same time, for higher-yielding varieties, no more than one parameter can be insignificant – i.e., the integrative attribute of yielding capacity is the result of interaction and mutual influence of at least six model parameters. While for the less yielding varieties the number decreases.

The analysis of grain quality was carried out for the following characteristics: protein content in the grain, gluten content in the grain, availability of high and low molecular weight glutenins in proteins and the total content of gliadins (Table 8). The first parameter is of key importance, as the protein content of 14% on average shows it can be referred to the class of strong wheat, which is of key significance for the baking industry. Thus, this class of materials includes varieties Podolyanka, Perlyna Podillia, Shpalivka, Zoryanka, Syla, Farell, NE 12443, Retezat, Ronin, Seilor, and Azano ($F=13.62$; $F_{0.05}=4.88$; $P=2.2*10^{-15}$). Of which, varieties Syla, Farell, Ronin, Seilor, and Azano, in turn, are more productive, therefore they can be recommended for growing, considering the combination of flour strength and high grain productivity. Podolyanka, Shpalivka, and Retezat have both productivity and quality at the standard level, and therefore, in general, are at the reference level for the region. While Perlyna Podillia, Zoryanka, and NE 12443 varieties are generally low-yielding and can be used, particularly the latter, exclusively as a source of material for breeding. Syla and NE 12443 were significantly positively distinguished as carriers of high quality and as potentially strong wheat ($F=8.17$; $F_{0.05}=3.55$; $P=0.01$).

In terms of gluten content, the pattern is about the same, since this indicator strongly correlates with the indicator of protein content. In general, it makes no sense to consider it separately. With regard to the compositions of protein components, high levels of high molecular weight glutenins and high content of gliadins should be attributed to positive qualities, while a high rate of low molecular weight glutenins is negative. Shpalivka, Zoreslav, Syla, Farell, NE 12443, Retezat, and Seilor ($F=8.34$; $F_{0.05}=5.11$; $P=0.01$) were significantly positively distinguished by the first feature, while Perlyna Podillia, Sonata Poltavska, Zoreslav, Syla, Farell, NE 12443, and Seilor ($F=7.16$; $F_{0.05}=4.55$; $P=0.03$) were negatively distinguished by the second attribute. It is known that this feature became noticeable in the negative aspect relatively recently and the necessary adjustments are being made to grain quality breeding programs. Moreover, this aspect influences nutritional value and possible allergic reactions rather than baking qualities. As for the indicator of the gliadines content, it is quite high in Sonata Poltavska, NE 12443, and Retezat, that is, it is extremely rare. All of the features, except for the low-variable content of gliadins are considered to be average, which is more favourable for sampling by these parameters. The pairwise comparison using Tukey's test confirmed these results. Thus, varieties Syla, Farell, Ronin, Seilor, and Azano were distinguished primarily by the combination of increased yielding capacity with advanced baking characteristics. Varieties Podolyanka, Shpalivka, and Retezat have yielding capacity and quality at an acceptable level, when taking into account the negative parameter of a high content of low molecular weight glutenins – varieties Ronin and Azano are preferable. However, they have lower gliadines content than other varieties. Thus, it is impossible to distinguish at least one variety that would excel others in terms of all parameters.

Table 8. Grain quality parameters (at average)

Variety	PC	GC	Glutenins		Gliadins
			HMW	LMW	
Podolyanka	13.97 ± 0.24[a]	25.44 ± 0.34[a]	0.16 ± 0.01[a]	0.46 ± 0.01[a]	0.44 ± 0.02[a]
Samara 2	13.48 ± 0.32[a]	22.98 ± 0.32[b]	0.18 ± 0.01[a]	0.54 ± 0.02[a]	0.42 ± 0.01[a]
Perspektyva Odeska	12.55 ± 0.27[b]	20.40 ± 0.29[c]	0.19 ± 0.01[a]	0.54 ± 0.02[a]	0.43 ± 0.02[a]
Perlyna Podillia	13.92 ± 0.22[a]	21.51 ± 0.22[b]	0.15 ± 0.02[a]	0.71 ± 0.02[b]	0.45 ± 0.01[a]
Sonata Poltavska	13.46 ± 0.16[a]	22.21 ± 0.28[b]	0.15 ± 0.01[a]	0.65 ± 0.01[c]	0.48 ± 0.01[b]
Shpalivka	14.14 ± 0.29[a]	26.69 ± 0.27[d]	0.20 ± 0.01[ab]	0.43 ± 0.02[a]	0.36 ± 0.02[c]
Zoreslav	13.42 ± 0.19[b]	24.72 ± 0.29[a]	0.22 ± 0.01[b]	0.58 ± 0.01[b]	0.40 ± 0.01[ac]
Grom	13.02 ± 0.31[b]	24.33 ± 0.31[a]	0.17 ± 0.01[a]	0.64 ± 0.01[c]	0.43 ± 0.01[a]
Zoryanka	13.71 ± 0.19[a]	19.85 ± 0.21[c]	0.16 ± 0.01[a]	0.64 ± 0.01[c]	0.45 ± 0.01[a]
Poradnytsia	13.66 ± 0.15[a]	22.14 ± 0.22[b]	0.15 ± 0.02[a]	0.70 ± 0.02[bc]	0.42 ± 0.01[a]
MIP Lada	13.11 ± 0.14[b]	23.02 ± 0.17[b]	0.17 ± 0.01[a]	0.66 ± 0.01[c]	0.43 ± 0.01[a]
Syla	14.46 ± 0.11[c]	25.78 ± 0.29[a]	0.23 ± 0.01[b]	0.70 ± 0.02[bc]	0.44 ± 0.01[a]
Farell	14.01 ± 0.26[a]	25.01 ± 0.32[a]	0.20 ± 0.01[ab]	0.45 ± 0.02[d]	0.39 ± 0.02[c]
Amandus	13.17 ± 0.24[b]	24.81 ± 0.27[a]	0.16 ± 0.01[a]	0.54 ± 0.02[a]	0.43 ± 0.01[a]
NE 12443	14.90 ± 0.23[c]	27.98 ± 0.25[d]	0.25 ± 0.01[b]	0.59 ± 0.02[b]	0.52 ± 0.03[b]
Retezat	14.18 ± 0.15[a]	26.19 ± 0.24[d]	0.24 ± 0.01[b]	0.47 ± 0.01[a]	0.48 ± 0.01[b]
Ronin	13.99 ± 0.19[a]	25.13 ± 0.23[a]	0.16 ± 0.01[a]	0.49 ± 0.01[a]	0.34 ± 0.03[c]
Patras	13.24 ± 0.20[b]	24.22 ± 0.24[a]	0.17 ± 0.01[a]	0.46 ± 0.01[a]	0.45 ± 0.01[a]
Seilor	14.11 ± 0.23[a]	24.09 ± 0.31[ab]	0.23 ± 0.01[b]	0.51 ± 0.01[a]	0.39 ± 0.01[c]
Azano	13.89 ± 0.22[a]	26.11 ± 0.26[d]	0.21 ± 0.01[a]	0.49 ± 0.01[a]	0.34 ± 0.02[cd]
Average	13.72	23.83	0.19	0.56	0.42
C.v, %	5.75	11.71	5.02	7.16	4.95

Note: PC – protein content [%]; GC – gluten content [%]; HMW – high molecular weight clutenins [%]; LMW – low molecular weight clutenins [%], significant differences at P<0.05 by factor analyze.

It should be noted that the selected varieties are referred to the genotypes of foreign breeding. The key problem for the Ukrainian varieties is the balance of reserve protein components, which never meets all the requirements in full. In general, however, the same varieties are technologically feasible. There is a significant negative correlation of high yielding capacity and improved quality, which cannot be found in foreign varieties. How-ever, promising donors have been identified by individual parameters, which makes it possible to obtain a breeding material with the necessary com-positions.

A key point for creating an efficient agrocenosis of any crop is the varietal component. The correct selection of varieties/combination of varieties in the production sowing ensures up to 30% of success (Tsenov *et al*, 2015). At the same time, the main point is not only the successful fulfilment of the genetically determined potential in terms of yield and quality, but also the stability in the manifestation of all the characteristics determining this potential throughout ontogenesis (Nuttall *et al,* 2017). Obtaining stable, predictable values is sometimes better than a problematic increase in the total grain amount or its baking qualities, its dietary value (Žofajová *et al*, 2017).

Characteristics that determine resistance to abiotic stress (in our case, winter resistance and drought resistance are the key ones) (Vesali *et al,* 2017; Avtaeva *et al,* 2021a; Pirych *et al,* 2021) should not only be stable, but also be effective in the required critical phases of development, that is the periods from January to February for winter varieties, requiring winter resistance, and from May to June (Masliev *et al,* 2020), for which drought resistance is essential (Wang et al, 2017). Finding new mechanisms to ensure efficiency of these features (such as a decrease in the consumption of accumulated sugars in the winter or high rates of photosynthetic activity during the heading stage) (Cann *et al,* 2022) allows to significantly stabilize and correlate over time the implementation of these properties, when the impact of an undesirable factor is most intensive (Quintero *et al*, 2018; Nazarenko *et al*, 2021).

Another option is to avoid this critical period through ontogenesis regulation (Nazarenko *et al,* 2022), however, within the studied set of varieties, the use of this mechanism has not been observed and, apparently, its role due to partial adverse effects time transfer due to global climate change has not been observed (Nuttall *et al,* 2017; Miroshnychenko *et al*, 2021). However, this does not mean a refusal of the cultivation of early maturing winter wheat varieties (Nazarenko *et al*, 2019), and additional research with a wider set of genotypes is needed, which in the past has shown that this method remains very significant (Wang *et al,* 2017; Beiko and Nazarenko, 2022b).

Modern varieties ensure stable increase in grain productivity at the appropriate level (Amram *et al,* 2015; Nazarenko and Izhboldin, 2017), however, it is quite often impossible to focus on this parameter only (Quintero et al, 2018). The materials presented indicate a number of shortcomings in terms of the grain quality, which still need to be eliminated, for which the necessary source material has already been allocated (Richardson *et al*, 2017).

Three possible ways to fulfil the yield potential have been identified: through the formation of a well-grained main ear with high-quality grain, which is quite common for contemporary breeding, due to the formation of additional quality ears (which is promising, although it provides for additional requirements for nitrogen nutrition of plants), and the third mixed possibility involves a particular ratio of blending both of the above mechanisms. It is also potentially possible to intensify this attribute due to the extension of the period of photosynthetic activity (Richardson *et al,* 2017; Beiko and Nazarenko, 2022a). However, this has not been observed yet during the phases (Jaradat, 2018). Potentially, the domestic varieties provide a sufficient degree of solving the problem of increasing the productivity of the agro-industrial complex in terms of a combination of productivity and quality (Hans *et al,* 2019; Miroshnychenko *et al,* 2021). Also, in general, a fairly large share of foreign varieties has been found, which, having been created for slightly different conditions, fully meets the needs of the zone of insufficient humidification (Harkness *et al*, 2020).

CONCLUSIONS

The use of various winter wheat genotypes (varieties) together allows, with a correctly selected combination, to cover the maximum possible changes in climatic conditions, which in principle has become important in recent years. The selection of specific varieties allows for the ecological test of the variety. And also helps to solve the problem of variety changing for the farms in the region. Despite the general mitigation of abiotic stresses, the work to increase the adaptive capacities of the source material still remains very relevant. At the same time, it is essential to study the peculiarities of the most critical phases in plants' development. In the future, it is planned not only to conduct research on other sets of soft winter wheat varieties, but also to detail the parameters of studying drought resistance by recording changes in photosynthetic activity, studying the nature of mechanisms of grain quality formation due to various combinations of alleles conditioning protein compositions and microelement composition.

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ARSENIC CONTENT AND MOBILITY IN AGRICULTURAL SOILS IN TWO POLLUTED AREAS IN KOSOVO

SUMMARY

Arsenic is a toxic chemical that can be found naturally in soil and rock. Exposure to high levels of arsenic in soil can have detrimental effects on human health, including cancer and skin damage. The presence of arsenic in the soil can be caused by both natural and human-made sources, such as mining and industrial activities. A total of 60 soil samples were collected in the agricultural soils of two regions of Kosovo known for pollution with heavy metals. Arsenic was extracted from the soil with aqua-regia (pseudo total concentration), NH_4OAc-EDTA (potential bioavailable), and NH_4NO_3 (mobile fraction). The pseudo total content of As showed a high value in Mitrovice up to 654.89 mg kg^{-1} with a mean of 66.51 mg kg^{-1}, whereas in Drenas region up to 23.63 mg kg^{-1} with a mean of 12.31 mg kg^{-1}. All samples analyzed in the area of Drenas were within the target values, while those of Mitrovica only 50% were within the target values, 30% within the accepted values and 20% above the values allowed for the content of arsenic according to the legislation. The rate of extraction of the potentially bioavailable and mobile form was low with a mean of 0.003 mg kg^{-1} in Drenas area and 0.2683 mg kg^{-1}, respectively 0.008 mg kg^{-1} in the Mitrovica area. The T test showed significant differences between regions in the three forms of arsenic content in agricultural soils.

Keywords: Potential bioavailable, mobile fraction, pollution, target values

INTRODUCTION

Arsenic is a naturally occurring toxic metalloid that is widely distributed in the environment. It can be found in soil, water, and air. Arsenic is often found in ores of silver, tin, lead, cobalt, copper and nickel (Krivokapić, 2020). In

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agricultural soils, arsenic can come from natural sources, such as weathering of mineral deposits, or from anthropogenic sources, such as the use of arsenic-containing pesticides and fertilizers (De Francisco *et al*., 2021; Singh and Srivastava, 2020; Yamamura and Amachi, 2014). In the USA, herbicides with Arsenic are allowed with strict rules, while in EU countries pesticides based on As are prohibited. Arsenic in agricultural soil can pose a threat to human health and the environment if it is taken up by crops and enters the food chain (He *et al.*, 2021).

Studies have shown that arsenic can accumulate in the roots, stems, and leaves of crops grown in contaminated soil (Cao *et al*., 2019). The extent of arsenic uptake by crops is dependent on various factors such as the chemical form of arsenic in the soil, soil pH, and the type of crop. The process of arsenic (As) uptake by plants from the soil is influenced by several factors, including the chemical forms of arsenic present in the soil, the specific plant species, and the overall concentration of arsenic in the soil. Generally, higher total soil arsenic concentrations lead to increased arsenic uptake by crops (Punshon *et al*., 2017).

Arsenic in agricultural soil can also affect the health of soil microorganisms and invertebrates, which play an important role in soil fertility and nutrient cycling (De Francisco *et al*., 2021). Arsenic toxicity can also lead to reduced growth and productivity of crops (Beniwal *et al.*, 2023), which can have significant economic consequences for farmers.

One of the major sources of heavy metals in Kosovo is the Trepça mine, which has been in operation for over 100 years. The mine produces a variety of minerals, including lead, zinc, and cadmium, which can contaminate the surrounding environment if not properly managed. Industrial activity and mining mostly cause pollution of the agricultural soils and environment, which are located in different parts of Kosovo. Zogaj *et al*. (2014) identified some hotspots in agricultural soils, e.g. field sites near the ore-metallurgic combine “Trepça” in Mitrovice, the ferronickel production plant “Ferronikeli” in Drenas, the battery Factory Ni-Cd in Gjilan and the mine of Kizhnica.

The management of arsenic in agricultural soil is a complex issue and requires a multi-disciplinary approach and in Kosovo there is insufficient investigation about arsenic and their suitability, especially in agricultural soils. It is important to understand the levels of arsenic in soil and take appropriate measures to minimize exposure to this toxic substance. Therefore, the aim of this research is to will provide an overview of arsenic and its mobility in potentially contaminated soils area in Kosovo.

MATERIAL AND METHODS

Study area

The areas of agricultural soils chosen for this study belong to the area of Mitrovica and Drenas, which are considered the most contaminated areas in Kosovo (Zogaj *et al.,* 2014; Maxhuni *et al*.,2023). The area of Mitrovica is located in the northern part of Kosovo and was one of the main industrial sites of

Former Yugoslavia and one of the most important mining districts in Europe (Nannoni *et al.*, 2011). Mitrovice Industrial Park (Trepca) included approximatly 40 mines, various concentrators, flotation and smelting plants, and several factories. This area has begun to be exploited intensively for the production of Pb, Zn, Au, Ag, and Bi from the 1930s, generating various categories of waste. Whereas the area of Drenas is located in the central part of Kosovo and the main industry is Ferronikeli factory, which is very close to agricultural and residential areas. Around 3 million tons of granulated slag is stored in a dump close to the factory.

Soil sampling and analysis

As shown in Figure 1., through the agricultural soils, 30 soil samples were taken per region (60 samples in total) at the plow layer, respectively 0-30 cm depth, according to the random method (BBodSchV, 1999; Theocharopoulos *et al.*, 2001). The soil samples were air-dried and then crushed and sifted to a size less than 2mm. Soil samples were kept at room temperature until analyzed. Soil pH was measured using a $CaCl_2$ solution with a ratio of 1:2.5 (DIN ISO 10390 2005). The total amount of carbon (C) and nitrogen (N) was determined using a C-N-S element analyzer (Elementar) based on gas chromatography. The particle size distribution was obtained by a combination of sieving and pipette methods after the removal of carbonates (HCl) and organic matter (H2O2) and dispersion in Na-pyrophosphate (ISO 14688 – 1: 2003-01 2003).

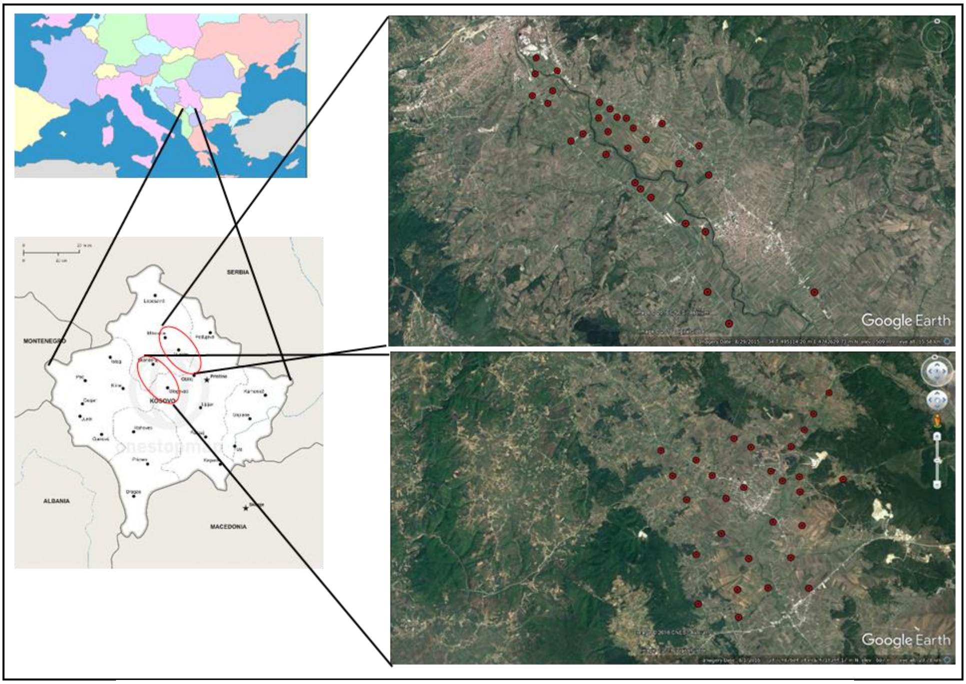


Figure 1. Location and soil sampling sites in the research areas

The method used to extract the pseudo total arsenic contents in soil involved the use of microwave assisted extraction (MAE) with aqua regia (a mixture of 35% hydrochloric acid and 65% nitric acid) from finely ground soil samples, in accordance with the US EPA 3051A method. However, this pseudo total content alone is insufficient to determine ecotoxicologically relevant arsenic. Thus, the exchangeable and mobile fractions of arsenic, which are potentially plant available and easily leachable, were extracted using NH4OAc-EDTA (ammonium acetate and ethylenediaminetetraacetic acid) and 1M NH4NO3, respectively, according to German regulation (DIN 19730:2009).

The arsenic concentrations in soil extracts were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES), the Varian 720ES model.

RESULTS AND DISCUSSION

The study involved the analysis of soil samples to investigate the factors affecting the behavior of arsenic in soil, specifically pH, clay, and carbon (C) content. The impact of two regions on the analyzed parameters was assessed using the Student t-test. Descriptive statistics indicated that the mean pH and clay content were similar between the two regions. However, the mean C content differed significantly between the regions (2.3 and 1.6), as confirmed by highly significant differences in the Student t-test ($p<0.001$) (table 1).

Table 1. The arsenic fraction content and other agricultural soils properties in samples analyzed (n=60)

		Mean	Median	STDEV	Min	Max	T-test
As$_{-AR}$	*Drenas*	12.31	11.74	3.23	8.37	23.63	-2.448**
(mg.kg^{-1})	*Mitrovica*	66.51	29.88	121.22	11.43	654.89	
As$_{-EDTA}$	*Drenas*	0.003	0.000	0.006	0.000	0.025	-2.196**
(mg.kg^{-1})	*Mitrovica*	0.268	0.031	0.662	0.000	3.538	
As$_{-AN}$	*Drenas*	0.003	0.003	0.003	0.000	0.014	-1.86*
(mg.kg^{-1})	*Mitrovica*	0.008	0.004	0.011	0.000	0.063	
pH	*Drenas*	6.07	6.09	0.73	4.54	7.38	0.955^{ns}
	Mitrovica	5.91	5.88	0.54	4.78	6.97	
Clay (%)	*Drenas*	38.03	35.06	12.97	17.22	68.45	-0.020^{ns}
	Mitrovica	38.09	37.78	8.4	22.55	57.28	
C (%)	*Drenas*	2.33	1.91	1.07	1.11	5.04	3.558***
	Mitrovica	1.61	1.6	0.28	1.18	2.22	
Limited value of soil contamination (As): A=30; B=55 and C=80 *mg.kg^{-1}*							

*As-AR – Aqua regia extraction; As-EDTA – EDTA extraction; As-AN - Aminium nitrat extraction; ns - no significant difference; *, **, and *** indicate significant differences at 5, 1, and 0.1% confidence level, respectively. A=clean; B=acceptable contamination, but further investigation are required; C=high contamination and needs to be cleaned*

Pseudotatal of arsenic in soil

The pseudototal of arsenic content in soil as shown in Table 1, in the Drenas area vary from 8.37 to 23.63 mg kg^{-1}, with a mean of 12.31 mg kg^{-1}. Whereas in the Mitrovica region the content of arsenic was highest with a maximum of 654.89 mg kg^{-1} and minimum 11.43 mg kg^{-1} (mean 66.51). Based on the Kosovo standard (Administrative instruction, 2018) for the level of heavy metals in the soil (the critical value for As 80 mg kg^{-1}), the results have shown that in the region of Mitrovica, 20% of the analyzed samples are above the permitted level or high contamination and needs to be cleaned these soils. Meanwhile, in the Drenas region, all analyzed samples were below the target level according to the Kosovo standard (30 mg kg^{-1}).

There is little data on the content of arsenic in the soils of the Drenas region, especially in agricultural soils, however, according to Imeri *et al.* (2019), in agricultural soils close to industry, the arsenic content was very low, only 0.007±0.001 mg·kg^{-1}. Our results have shown that the content of arsenic is significantly higher, this is probably due to the more advanced technique that we have used for the analysis of soil samples.

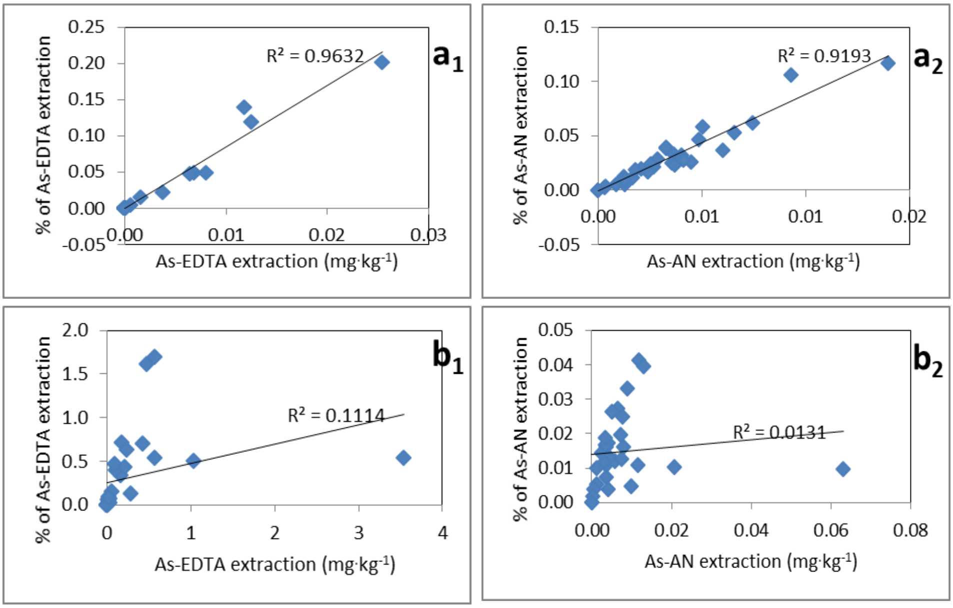
Such a high amount of arsenic in the soil in the area of Mitrovica is a consequence of the mining and processing activity that took place in this area in the past. Other authors have also reported high levels of arsenic in soils around mining areas, such as in the region of Freiberg, Germany, around 970 km^2 have been defined as contaminated with arsenic, where over 50% of the soil has shown a value above 55 mg kg^{-1} (Loukola-Ruskeeniemi *et al.*, 2022). Also near Verdun, France, studies have shown high levels of arsenic ranging from 15 mg kg^{-1} in the reference zone to 775 mg kg^{-1} in the highly contaminated site (Loukola-Ruskeeniemi *et al*., 2022).

In the area of Mitrovica according to the research of Stafilov *et al.* 2010, about 124 km^2 is above the target value according to the standard of Kosovo (30 mg kg^{-1}), while 64km^2 was above 55mg kg^{-1}, and which, according to the Kosovo standard, must be constantly monitored. As a result of the developed human activities in this area, As content at different levels has been reported from 2.1 to 3900 mg kg^{-1} (Aliu *et al*., 2019). Also, Barać *et al.* (2016) reported arsenic levels in floodplain agricultural soils along Iber River vary from 15 to 473 mg kg^{-1}. Student T-test has shown statistical significance in the pseudo-tatal content of arsenic in the researched areas ($p<0.01$) (table 1).

Bioavailability and mobile form of Arsenic in soil

The potential bioavailability form of arsenic in agricultural soils is given in graphic 1. As can be seen from the graph, the largest amount of As was extracted in the area of Mitrovica with 1.7% (graph 1 b), while in the area of Drenas this amount reached up to 0.2% (graph 1 a). The extracted mobile amount of As in agricultural soils showed higher values in the Drenas region with a maximum of 0.117 % (graph 1 a) compared to the Mitrovica region where this value only reached 0.041 % (graph 1 b). Similar results for the researched region have been reported by other authors, such as Barać *et al*. (2016); Nanoni *et al.* (2011).

Student T-test has shown statistical significance in the bioavailability and mobile form of arsenic in between the researched areas ($p<0.01$ and $p<0.05$ respectively) (table 1).



Graphic 1. The extracted amount of bioavailability of arsenic from the total in percentage and mg·kg^{-1} (a_1-potential bioavailability of As in Drenas region; a_2-mobile form of As in Drenas region; b_1-potential bioavailability of As in Mitrovica region; b_2-mobile form of As in Mitrovica region)

As shown in Table 2, the forms of arsenic showed a high correlation of 95% between As-AR and As-EDTA and As-EDTA with As-AN, respectively 91% between As-AR and As-AN. While their correlation with soil parameters has been weak.

Table 2. Correlation between forms of Arsenic and other properties

	pH CaCl2	*Clay %*	*N, %*	*C, %*	As-AR	*As-EDTA*	*As- AN*
pH CaCl2	1						
Clay %	0.40752985	1					
N, %	0.49249711	0.3982004	1				
C, %	0.46487709	0.4037168	0.975366	1			
As-AR	0.01374274	-0.219468	-0.059385	-0.118140	1		
As-EDTA	0.05698793	-0.225327	-0.052818	-0.102330	0.95262889	1	
As- AN	0.15932609	-0.167231	0.091207	0.036003	0.91727208	0.95580905	1

CONCLUSIONS

In the region of Drenas, all the analyzed samples were within the target values, while in the Mitrovica region, 50% of the analyzed samples were within the target values, 30% were within the accepted values but which require further research and 20% of the samples were above the allowed values for the content of arsenic in agricultural soils.

High concentration of arsenic has been noticed close to the sources of pollution, sometimes exceeding the allowed values several times. Since these lands are used for agricultural production, such a high amount of arsenic can be dangerous for human health and other organisms.

The potentially bioavailable and mobile form of arsenic has shown low extraction rates, however, due to the very high correlation of these forms with the total amount of arsenic, it can enter the food chain and may cause a health risk. The content of arsenic in the agricultural lands of the two regions has shown significant differences between them.

We recommend to the authorities responsible for land management to make a plan for soil remediation, especially in areas with high levels of arsenic. This can be achieved through phytoremediation using hyper accumulating plants such as sunflower, sorghum, lupine, etc. This can also be combined with bioremediation, namely the use of microbiological processes to convert arsenic into a less toxic form. We suggest also to farmers in these lands to produce plants that do not accumulate arsenic.

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HEAVY METALS ACCUMULATION AND DISTRIBUTION PATTERN IN MAIZE PLANTS

SUMMARY

This paper investigates the pattern of heavy metal accumulation and distribution in maize plants grown in a field near Sarajevo International Airport. Soil and maize plant samples from the studied soil were analyzed for selected heavy metals, i.e., chromium (Cr), cadmium (Cd) lead (Pb), zinc (Zn), copper (Cu) nickel (Ni) and manganese (Mn). The pattern of their accumulation and distribution were assessed using bioaccumulation factor (soil-root transfer) and translocation factor (root-leaves transfer). In this study, all the heavy metals studied (except Zn and Mn) exhibited higher contents in the analyzed root tissues than in the above-ground parts of maize (stem, leaves and kernels), suggesting that maize plants have versatile mechanisms to restrict transport of toxic heavy metals Cr, Cd, Pb, Cu and Ni from root to other parts of maize plants. On the other hand, Zn and Mn were more efficiently transferred from the maize roots to leaves. These results were expected, since Zn and Mn play an important role in various metabolic processes in plants. The study results also indicate that maize plants have a strong ability to absorb Cd ions from soil and accumulate them in roots, suggesting that maize is an efficient plant in phytostabilizing Cd.

Keywords: bioaccumulation, root-leaves transfer, toxic heavy metals, translocation

INTRODUCTION

Maize (*Zea mays* L.) is a member of Poaceae family, originally from Central America. It was introduced into Europe at the end of the 15th century, from where it spread through the Old World. It is now one of the most important and widely-grown cereal crops worldwide. Maize is not only an important food and feed crop, but also an important raw material for the manufacture of many industrial products, including maize starch, oil, syrup and biofuels. Global

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demand for maize products is therefore expected to rise worldwide in the future (Revilla *et al.,* 2022). To achieve it's ever rising demand for maize products, farmers apply different agricultural practices including among others the use of enormous amounts of chemical pesticides and fertilizers that can pollute soils, groundwater and the atmosphere (Tudi *et al.,* 2021). Natural sources can also increase soil heavy metal pollution including soil parent materials, volcanic eruptions, and rock weathering. In any case, heavy metals in excess amounts in soils lead to reduction in food quality and yields and can cause serious effects to human health through the food chain (Alengebawy *et al.,* 2021). Therefore, food security, particularly in the context of important crops such as maize, has become a global concern.

Many studies have reported that the effects of heavy metals on plant growth and development greatly depend on soil physicochemical properties and both the form and concentration of heavy metals in the soil (AL-Huqail *et al.*, 2022; Xu *et al*., 2022). However, there are relatively few studies investigating the physiological mechanisms of plant responses to heavy metal toxicity. In this light, plants have developed different strategies to overcome elevated levels of heavy metals in soils during evolution (Sladkovska *et al.*, 2022). The best-first strategy is to minimize heavy metal uptake from the soil through root exudates, such as oxalic acid or citric acid, which have a high affinity to form stable heavy metal complexes in the rhizosphere. In addition, some root exudates have the capacity to change the pH of rhizosphere, leading to precipitation of heavy metals, thereby limiting their availability to plant roots (Yan *et al.*, 2020). At next stage, if these strategies fail and heavy metals enter the root, plants may activate different tolerance mechanisms for heavy metal detoxification. This includes among others the low translocation of heavy metals from roots to the aerial parts, the embedding of heavy metals in the plant cell walls as well as chelation and compartmentalization of heavy metals in different intracellular compartments such as vacuoles where they are stored without toxicity (Emamverdian *et al.*, 2015). Selection of strategy to cope with heavy metal toxicity primarily depends on plant genetic background and on environmental factors that affect plant growth (Viehweger, 2014).

The current study was designed to study the effect of soil characteristics and its heavy metals content on heavy metal accumulation and detoxification mechanisms in maize plants. A better understanding of these mechanisms can help farmers and scientists to predict and explain the effects of heavy metals on maize production. Understanding these mechanisms also creates a predisposition to make the right decision about the possibilities of maize production on soils contaminated by heavy metals.

MATERIAL AND METHODS

Study area

This study was carried out in 2022 at the agricultural experimental station of the Faculty of Agriculture and Food Science located near Sarajevo

International Airport (Bosnia and Herzegovina). The site is located at 43°49′34.42″ N and 18°19′18.48″ E, at an altitude of 505 m above sea level (Figure 1).

Figure 1. Location map of the study area

The study area has a continental humid climate typified by cold and not so humid winters and warm and humid summers, with a mean annual temperature of 9.6 °C and a mean annual precipitation of 899 mm. This climate is classified as Dfb (warm-summer humid continental climate) according to the Köppen-Geiger climate classification (Kottek *et al*., 2006).

The experimental area is divided into three soil plots. Each plot was 3 m wide by 10 m long consisting of 4 rows. After preparing the soil for sowing, the domestic maize hybrid: BL 43 was sown (5 May 2022), maintaining 75cm×20cm plant spacing. The experimental field was fertilized with 180, 100 and 150 kg/ha of N (nitrogen), P (phosphorus) and K (potassium), respectively. The sources of N, P and K were urea, superphosphate and potassium chloride, respectively. N was applied in three equal splits, i.e. one day before sowing, 30 days and 60 days after sowing. The entire amounts of P and K were applied one day before sowing. Weeding was done manually as and when necessary during maize cultivation. Control measure for pests and diseases was not applied in this study.

Plant material

BL 43 is a dent type, mid-ripening and high-yielding maize hybrid. It is tolerant to drought and resistant to major maize diseases and leaf pests and aphids. It has very strong and elastic stalks, resistant to lodging and breakage. The ear is cylindrical, with 16-18 rows of kernels, while the grain is yellow and large, tightly attached to the cobs and has excellent quality.

Soil sampling and analysis

For the current study, soil samples from field site (the surface soil layer at a depth of 0-30 cm) were collected one month before the maize sowing (2 April 2022) using stainless steel spoon auger and then analyzed using standard soil testing methods. pH was measured in 1 mol/L potassium chloride solution using the LLG-7 pH Meter (solution-soil ratio 2.5:1). Organic matter was determined by oxidation method with potassium dichromate (ISO 14235, 1998), total N content by Kjeldahl method (Bremner, 1960), and the available P and K content by Egnér-Riehm method (Egnér *et al.,* 1960). The contents of heavy metals in soil samples were determined by atomic absorption spectrophotometry, after digestion of the samples with aqua regia solution (mixture of nitric acid and hydrochloric acid in a ratio of 1:3).

The digestion of soil samples for heavy metals analysis was done as follows: 3 g of air-dried soil sample was carefully weighed and transferred into a 250 mL flat bottom flask, after which 28 mL of aqua regia solution was added. The flask was covered with a watch glass and left for 16 h at room temperature in a fume hood. Then, the flask was connected to the reflux system and refluxed on a hot-plate for 2 h. After cooling to room temperature, the mixture was filtered through Whatman No.42 filter paper into a 100 mL volumetric flask and diluted to the mark with deionized water (ISO 11466, 1995).

The contents of investigated heavy metals in digested soil samples were determined using an atomic absorption spectrophotometer (Shimadzu AA-7000, Japan) according to the instructions specified in the ISO 11047 method (ISO 11047, 1998). The quantification of investigated heavy metals was carried out using an external calibration curve. The external calibration curves were prepared by diluting the stock solutions of 1000 mg/L of each element supplied from Merck (Darmstadt, Germany). All calibration curves were characterized by a high correlation coefficient ($r>0.995$).

Plant sampling and analysis

Three maize plants (including root, stem, leaves and kernels) from each experimental soil plot were carefully collected at the stage of full maturity. Leaves, stem, root, and fruits/kernels of each plant were separated, dried at room temperature, grinded and then stored in paper bags until analyses. The contents of heavy metals in plant samples were also determined by atomic absorption spectrophotometry, after wet digestion of the plant sample with mixture of nitric-perchloric acid in a ratio of 2.5:1.

The digestion of plant sample for heavy metals analysis was done as follows: 1 g of air-dried plant sample was carefully weighed and transferred into a 100 mL flat bottom flask, after which 10 mL of nitric acid and 4 mL of perchloric acid was added. The flask was covered with a watch glass and left for 2 h at room temperature in a fume hood. Then, the flask was gently heated on a hot plate for 30 min. After cooling to room temperature, the mixture was filtered through Whatman No.42 filter paper into a 50 mL volumetric flask and diluted to the mark with deionized water (Lisjak *et al*., 2009).

Bioaccumulation and translocation factor calculation

Bioaccumulation factor (BAF) is defined as the ratio of heavy metal content in plant roots to the soil. It was calculated by dividing the heavy metal content in the root sample by the heavy metal content in the soil sample. Translocation factor (TF) explains an ability of a plant to translocate the heavy metal from roots to above-ground parts of plant. In this study, it was calculated by dividing the heavy metal content in the leaf sample by the heavy metal content in the root sample of maize (Ahmed and Slima, 2018).

$$BAF = \frac{\text{C roots (heavy metal content in the root)}}{\text{C soil (heavy metal content in the soil)}} \quad (1)$$

$$TF = \frac{\text{C leaves (heavy metal content in leaves)}}{\text{C roots (heavy metal content in roots)}} \quad (2)$$

According to BAF values, the plants can be classified into four categories, namely no phytoaccumulation (BAF<0.01), low phytoaccumulation (0.01-0.1), moderate phytoaccumulation (0.1-1) and high phytoaccumulation (BAF>1) (Sekabira *et al*. 2011). In general, the plant species with BAF values >1 and TF values <1 have the potential to be used in phytostabilization, whereas plant species with both BAF >1 and TF >1 have the potential to be used for phytoextraction (Mohotti *et al*., 2016).

Statistical analysis

All analyses were carried out in triplicate and the results were expressed as mean value ± standard deviation. Analysis of variance and significance of difference among means were tested by one-way ANOVA and least significant difference (LSD) using the Microsoft Excel 2010 package program (Office 2010, Redmond, WA, USA). Statistical significance was considered with a 95% confidence interval (P<0.05).

RESULTS

Heavy metal contents and basic chemical properties of studied soil

Heavy metal contents in studied soil plots varied from 24.12 to 24.20 mg/kg for Cr, 0.29 to 0.30 mg/kg for Cd, 39.13 to 39.50 mg/kg for Pb, 37.11 to 38.22 mg/kg for Zn, 20.11 to 20.63 mg/kg for Cu, 34.11 to 35.01 mg/kg for Ni, and 622.66 to 697.31 mg/kg for Mn. The total contents of Cr, Cd, Pb, Zn, Cu and Ni did not exceed the proposed heavy metal limits for agricultural soils in Bosnia and Herzegovina (80, 1, 80, 150, 65 and 40 mg/kg, respectively). Limit value of Mn in soils is not established by legislative (OG FBiH, 2009) since it is not considered as directly contaminating element. However, the content of Mn in examined soils was lower than toxic level of Mn in soils (850 mg/kg) reported by Pais and Jones (1997).

Results obtained from soil analysis also showed that the examined soil plots have a slightly acid reaction (from 6.0 to 6.2), moderate level of organic carbon (from 3.0 to 3.2), and low content of available P (from 6.0 to 7.1) and K (from 9.1 to 12.2). The studied soil has a clay loam texture with a good balance between air-porosity and water-holding capacity, and is therefore considered ideal for maize production. According to World Reference Base for Soil Resources (IUSS, 2015), the studied soil can be classified as Alluvium. Alluvium soils develop on alluvial deposits near the river and are mostly characterized by a weak surface horizon and by parent material derived from river.

Heavy metal contents in maize plants

Heavy metals contents (Cr, Cd, Pb, Zn, Cu, Ni and Mn) in different parts of maize plants are presented in Table 1.

Table 1. Heavy metal contents in the maize plant samples

The parts of maize plant	Heavy metals (mg/kg dry mass)						
	Cr	Cd	Pb	Zn	Cu	Ni	Mn
root	6.34 ± 0.99[a]	1.23 ± 0.21[a]	0.09 ± 0.09[a]	37.1 ± 19.4[a]	28.9 ± 7.1[a]	14.7 ± 7.9[a]	43.2 ± 12.7[b]
stem	1.10 ± 0.21[b]	0.22 ± 0.13[b]	0.02 ± 0.05[b]	20.9 ± 11.6[b]	7.2 ± 2.6[bc]	1.16 ± 1.2[b]	9.8 ± 10.3[c]
leaves	1.17 ± 0.19[b]	0.27 ± 0.11[b]	0.02 ± 0.04[b]	39.2 ± 17.7[a]	12.5 ± 8.1[b]	1.44 ± 0.7[b]	77.9 ± 20.8[a]
kernels	0.62 ± 0.11[b]	0.10 ± 0.04[c]	0.01 ± 0.01[b]	20.1 ± 6.1[b]	3.1 ± 2.1[c]	1.13 ± 0.8[b]	6.1 ± 1.7[c]
$Lsd_{0.05}$[1]	0.59	0.13	0.05	14.41	5.66	3.67	13.43

[1]*Averages denoted by the same letter in the same column indicate no significant difference (P<0.05)*

The results showed that the contents of all examined heavy metals in the kernels i.e. edible parts of maize were below the maximum permissible value of heavy metals in food crops set by FAO/WHO (2001). Accordingly, the maximum permissible contents of Cr, Cd, Pb, Zn, Cu and Ni in the edible parts for human consumption are 2.3 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 100 mg/kg, 40 mg/kg and 4 mg/kg dry mass, respectively. The maximum permissible value for Mn in edible plant parts is not reported by FAO/WHO since Mn has relatively low toxicity for human health. The obtained results also showed that the content of Cr, Cd, Pb, Cu and Ni was significantly higher in the roots than in other parts of the maize plants.

Translocation of heavy metals from soil to maize roots and from roots to leaves of maize

The values of bioaccumulation factor (soil-root transfer) and translocation factor (root-leaves transfer) for the investigated heavy metals in the maize plants are presented in Table 2.

Table 2. Bioaccumulation and translocation factor values for the investigated heavy metals

Soil plot		Cr	Cd	Pb	Zn	Cu	Ni	Mn
1	BAF[1]	0.25	4.43	0.003	0.77	1.44	0.93	0.08
	TF[2]	0.20	0.20	0.27	0.98	0.50	0.10	1.69
2	BAF	0.29	3.83	0.002	0.88	1.59	0.65	0.06
	TF	0.14	0.23	0.14	1.28	0.48	0.11	1.72
3	BAF	0.25	4.13	0.001	1.21	1.22	0.59	0.06
	TF	0.22	0.12	0.17	1.01	0.32	0.09	2.01

[1]*Bioaccumulation factor (soil-root transfer) value*
[2]*Translocation factor (root-leaves transfer) value*

The bioaccumulation values (BAF) for the investigated heavy metals from soil to maize roots decreased in the order: Cd>Cu>Zn>Ni>Cr>Mn>Pb. The BAF value for Cd was much higher than 1, suggesting that the maize plants are good candidates for phytostabilization of Cd-polluted soil. Present study also showed that the translocation value (TF) was highest for Mn, followed by Zn and Cu, indicating that maize plants have a natural ability to translocate these elements from roots to above-ground parts.

DISCUSSION

Soils polluted by heavy metals can have unfavorable effects on plant growth and productivity. Moreover, heavy metals at toxic levels in soils can pose a significant health risk to food crops and consequently to humans via the food chain. To overcome elevated levels of heavy metals in soils, plants have evolved numerous detoxification mechanisms (Dalvi and Bhalerao, 2013). However, different plant species activate different detoxification mechanisms at physiological, biochemical and molecular levels, which mainly depend on plant genotype, soil properties and heavy metal concentration and availability in soils (Gill *et al.,* 2022). An understanding of these interrelated detoxication mechanisms in plants is important to predict the possibilities of plant growth on soils polluted with heavy metals.

In this study, all the heavy metals studied (except Zn and Mn) exhibited higher contents in the analyzed root tissues than in the above-ground parts of a maize plant. This finding strongly suggested that maize plants have versatile mechanisms to restrict transport of toxic heavy metals Cr, Cd, Pb, Cu and Ni from root to other parts of maize plants. Some of these mechanisms could be as follows: (1) heavy metal ion binding to the cell wall, (2) chelation of heavy metals in the cytosol with strong ligands, (3) heavy metal sequestration in the specific root cells and (4) efflux of heavy metal from the cytosol of a root cell, either into the apoplast or into the vacuole. The metal translocation restriction phenomenon for maize plants was most pronounced for Ni, followed by Cr, Cd and Pb. This result was expected, since above-mentioned heavy metals have no known physiological function in plants. Moreover, they are toxic for plants even at very low concentrations (Jaishankar *et al.,* 2014).

In this study, Zn and Mn were more efficiently transferred from the maize roots to leaves as compared with Cr, Cd, Pb and Ni. Similar results were found by Shafiq *et al.* (2020) and Abedi *et al*. (2022). These results were also expected since both Zn and Mn play an important role in various metabolic processes tightly associated with photosynthesis and therefore the plant tends to translocate them to the leaves (Hänsch and Mendel, 2009). Among others, Mn participates in the structure of photosynthetic proteins (Alejandro *et al.,* 2020), while Zn acts as a cofactor of numerous enzymes involved in photosynthesis and sugar formation (Umair Hassan *et al.,* 2020). In the light of the above mentioned, it is obvious that heavy metal distribution within plant differs considerably, primarily depending on the heavy metal toxicity and their role in plant metabolism. Numerous studies have also confirmed this observation (Angulo-Bejarano *et al.,* 2021; Riyazuddin *et al.,* 2021).

In the present study, the BAF value for Cd was the highest one. This value was much higher than 1 (ranged from 3.83 to 4.33), suggesting that maize is a promising species for phytostabilization of Cd-contaminated soil. The obtained results are in an agreement with those of Li *et al.* (2012) and Rizwan *et al.* (2017). Huang *et al.* (2020) reported that Cd uptake by root cells occurs through plasma membrane transporters involved in the uptake of essential elements such as Ca, Mg, Fe and Zn. It is therefore not surprising that many plants, including maize, can easily absorb Cd from the soil (Sun *et al.,* 2022). However, the maize plants have the strong ability to prevent or slow down the Cd translocation from roots to above-ground parts, including the grain. This finding indicates that maize can grow healthy on soils contaminated with certain level of Cd. This hypothesis has, in fact, been confirmed by many other scientists (Seifikalhor *et al*., 2020; Sterckeman and Thomine, 2020).

This study indicated that the BAF value for Zn and Cu in maize plants was also at a higher level. Taking into account that both Zn and Cu are essential for plant metabolism, these results were as expected.

The BAF value for Pb was the lowest (ranged from 0.001 to 0.003), suggesting that maize plant roots can uptake only small amounts of Pb from the studied soil. This data is highly desirable, since the Pb is one of the most toxic metals for living organisms, even when absorbed in small amounts (Collin *et al.,* 2022). However, this finding is not consistent with the findings of several studies where the values of Pb soil-root transfer for maize and other plants were much higher (Aladesanmi *et al*., 2019; Chiwetalu *et al*., 2022). It is obvious that the Pb uptake by roots differs significantly among different plant species and even within a species (Bassegio *et al*., 2020). It depends primarily on soil chemical properties, including among others, pH, organic carbon content, redox potential and cation exchange capacity (Luo *et al*., 2014). Soil microbes could also affect Pb mobility and availability to plant roots mainly through alteration of soil pH (Gladkov *et al*., 2023). The pH value of studied soil was found to be slightly acidic, which is not favorable for release and mobility of Pb in soil. This is probably one of the most important reason for low Pb mobility in the analyzed soil and consequently for low Pb transfer from soil to maize roots. Namely, Pb is more soluble in acidic soils than in slightly acidic, neutral and alkaline soils, where Pb precipitates as hydroxides and carbonates, rendering it less accessible to plants (Li *et al.*, 2021).

From the health point of view, an interesting finding is that the maize grown in the studied soil accumulate lower levels of toxic heavy metals (Cr, Cd, Pb and Ni) in kernels than in the other parts. Moreover, the contents of above-mentioned heavy metals in kernels i.e. the most edible part of the plant were much lower than the permissible limit set by FAO/WHO (2001). Similar findings were reported by Zhou *et al.* (2022), indicating that maize relieve health risks of heavy metal-polluted soils. Future studies will be needed to verify this hypothesis.

CONCLUSIONS

Overall, the results of the present study indicate that maize plants have an ability to reduce Pb uptake from soil to roots as well as to restrict transport of toxic heavy metals Cr, Cd, Pb, Cu and Ni from root to above-ground parts under experimental conditions. The study also showed that maize plants have a great potential to absorb Cd ions and accumulate them in roots, suggesting that maize is an efficient plant in phytostabilizing Cd. In this study, the accumulation pattern of all tested heavy metals in maize organs was arranged as: roots>leaves>stem>kernels. Also, the levels of all heavy metals, in maize grains, were lower than the permissible limits set by FAO/WHO, indicating that their translocation to the edible part was comparatively low or absent. In general, the study highlights that the consumption of maize grown on studied soil, from the point of view of heavy metals, should not be dangerous to human health, suggesting that maize relieve health risks of heavy metal-polluted soils. However, further investigations are needed to confirm this hypothesis.

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CHANGES IN RIVER BANK MORPHOLOGY IN A SMALL MEANDER OF EL ABID RIVER, ATLAS MOUNTAINS, MOROCCO

SUMMARY

This paper presents initial findings stemming from an extensive and first-hand field monitoring endeavour that delves into the erosion of a fluvial section in the downstream part of Oued El Abid. This river, situated within the Central High Atlas, holds particular prominence as the primary tributary of the big Oum-Err-Bia River. The hydrological behaviour of the El Abid River follows a snow-rainfall pattern, characterized by an average monthly precipitation of 82 mm. The main purpose of the current study is to investigate how the El Abid River responds to the forces of fluvial dynamics, particularly in the downstream segment near the Bin El Ouidane dam, by employing a methodology cantered on Geographic Information Systems (GIS) and Google Earth imagery spanning from 2016 to 2022. The findings of the study reveal a significant metamorphosis and ongoing dynamism within the targeted meandering section at the river's outlet. The period of research from 2016 to 2022 has witnessed pronounced alterations driven by lateral activities and a spectrum of fluvial processes: erosion, transportation, and deposition. This continual fluvial erosion has culminated in substantial soil loss from the riverbanks. Inevitably, these transformations intensify the sedimentation and siltation processes downstream, directly impacting the Bin El Ouidane reservoir as an outcome of the El Abid River's discharge dynamics.

Keywords: Fluvial erosion; Morphology; El Abid river bank; Atlas Mountain; Morocco

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INTRODUCTION

In recent times, there has been a noticeable surge in research attention directed towards the dynamics of fluvial bank erosion (Konsoer *et al*., 2016). This trend can be attributed, in part, to several factors: (i) An increasingly heightened recognition of the pivotal role that bank erosion plays in influencing the dynamics of fluvial sediment transport, the sediment yields within basins, and the creation and destruction of floodplains (Billi and Spalevic, 2022); (ii) A growing emphasis on studies pertaining to channel stabilization, within which the analysis of bank failures holds a central position; (iii) Notable advancements made within the last decades in models (Darby and Thorne, 1996) and monitoring techniques (Lawler, 1991, 1994). These advancements have significantly contributed to addressing some of the persisting challenges (Coulthard *et al.*, 2012) and challenges include understanding the dynamics of bank erosion events, pinpointing the mechanisms driving bank retreat, defining intricate combinations and sequences of processes, with establishing connections between the supply of sediment from the banks and the transport of sediment within the channel (Lawler *et al*., 1999). Experiences of similar studies are identifying usually five distinct approaches used to examine the progression of meandering channels (de Vente *et al*., 2008; Gao, 2008; Milewski *et al.*, 2020; Vojtek *et al*., 2019), including three categorized as methodologies concentrating on meander morphology (Magdaleno and Fernández-Yuste, 2011). These methodologies involve scrutinizing the channel's characteristics over a specific time period. The other two approaches are focused on directly observing alterations experienced by the river's planform (Hooke, 1984). Consequently, given the extensive array of environmental and historical changes in processes within a basin's evolution, it becomes unfeasible to precisely predict or possess complete knowledge regarding the alterations experienced by a channel (Constantine *et al.*, 2009; Duan and Julien, 2005).

Several research endeavours have pointed out the limitations of traditional hydraulic viewpoints, such as regime theory or other geometric geomorphic approaches, in accurately predicting the consequences arising from changes in the environment within river systems. Factors like the scale of operations, the degree of alterations, and the spatial arrangement and spread patterns emphasize that the hydrological response and behaviour of river systems don't solely depend on external factors, or at least are not limited to them entirely (Latron *et al*., 2009; Latron and Gallart, 1995). Instead, it hinges on the landscape and the structural arrangement of the system, shaped by its geomorphologic history. This, in turn, reveals a non-linear aspect of how river systems react to modifications in extraneous conditions (Downs and Gregory, 2004).

The objective of this study is to continuously monitor the evolution of the morphology of a meander in the downstream part of the Oued El Abid River within the Central High Atlas of Morocco with an assessment of the meandering process that has been monitored since 2016 to investigate the impact of fluvial erosion on the river bank of the studied section.

MATERIAL AND METHODS

Study area

Positioned within the Central High Atlas of Morocco, Oued El Abid River emerges as a significant tributary of the Oum Err Bia River, as presented in Figure 1. Administratively, this river belongs to the region of Beni-Mellal - Khenifera, and the province of Azilal (Ouakhir *et al.,* 2022).

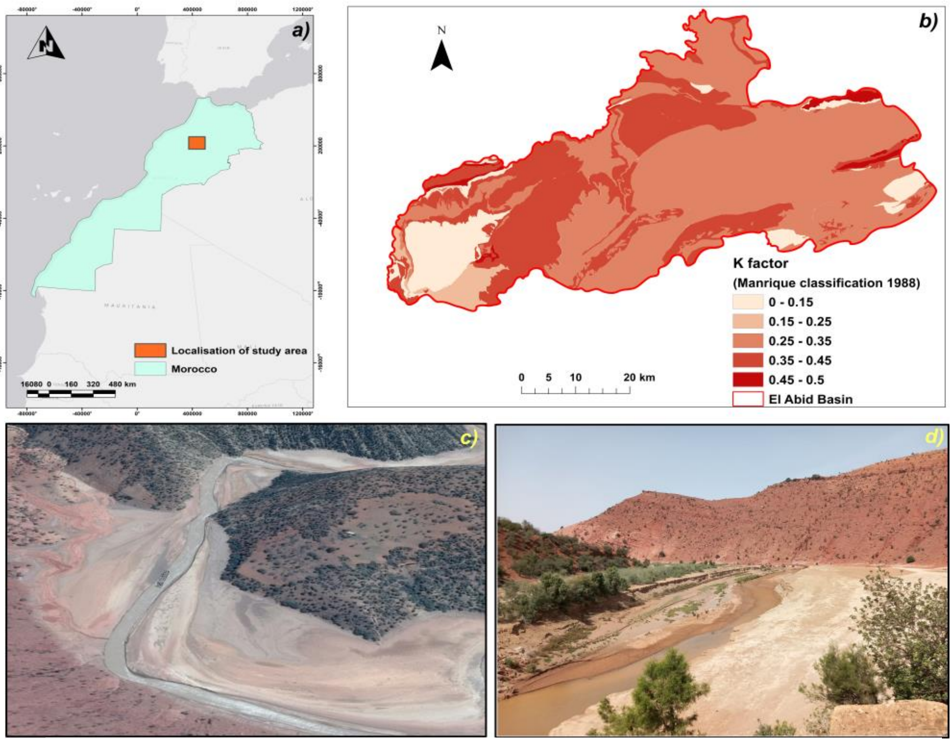

Figure 1. a) Position of Morocco in the North Africa, b) El Abid River basin with presentation of Erodibility factor (K); c) the Google Imagery of 2016 ®, and d) photo from the field of the studied meander of El Abid river (Source: Original, 2023).

The climate in the upper part of the basin leans towards semi-arid, characterized by the concentration of precipitation during the autumn and winter seasons. This climatic attribute yields a torrential regime that accelerates sediment transport and leads to silt accumulation downstream, affecting the Bin El Ouidane dam (Ouakhir *et al.*, 2023), as presented in Figure 1 b, c. Temperatures reach 35°C in August, while a low temperatures of –5°C are documented in January (Karroum *et al*., 2019). The geographical area of El Abid basin is nestled between the mountainous northern coastline: The Rif Mountains; and the interior: the Atlas Mountains (Karaoui *et al*., 2017). This territory is bordered by wide plateaus interspersed with valleys and fertile coastal plains. The mean elevation of this terrain stands at 909 m a.s.l, with the highest altitude being Jebel Toubkal at 4165 m a.s.l. Limestone, marl, and clay outcrops constitute the prevailing lithology of the basin, coupled with a substantial quaternary deposit evident along the banks of the El Abid river (Ouakhir *et al*., 2019).

El Abid basin holds a mountainous character, adorned with meadows and pastures. Notably, the frequency and intensity of floods have experienced a noteworthy upsurge in the study area over recent decades (Cherifi and Loudiki, 1999). This surge can be attributed to a combination of factors, including heightened rainfall intensity, alterations in land use (urbanization, vegetation changes, deforestation), and the rugged topography typified by steep slopes (Barakat *et al*., 2016). The analyzed basin's K factor (Figure 1b) demonstrated a significant vulnerability of soil to detachment and erosion because of the intensity of rainfall and runoff (Ouakhir *et al*., 2022). Furthermore, the spatial arrangement of mean soil erosion within the El Abid river basin between 2000 and 2022 exhibited a consistently elevated pattern in terms of annual average soil erosion, surpassing 20 t ha^{-1} yr^{-1} and covering 62.6% of the entire area. Notably, fluvial erosion held a particularly prominent role in this scenario (Ouakhir *et al.,* 2022).

Material

Total topographic station was used to take the topographic profiles in order to monitor the fluvial dynamic and detect the change in river banks of the studied section (Figure 2).

Figure 2. Total topographic station used for extracting topographic sections

This sophisticated surveying instrument used combines the functions of a theodolite and an electronic distance measuring (EDM) device, allowing us to accurately measure angles and distances in three dimensions (horizontal, vertical, and slope) to create detailed topographic maps and profiles. This allows us to record measurements directly into the instrument and process the data later on a computer to create maps, profiles, and other graphical representations; provided geodetic coordinates (latitude, longitude, and elevation) for surveyed points. This was crucial for creating accurate topographic maps and for precise positioning of objects or features on the surface of the studied region.

Methods and data

The investigation into the downstream meandering of the Oued El Abid River has commenced since 2016, focusing on the comprehension of the interactions and dynamics of fluvial erosion processes. To achieve these aims, distinct profile sections were surveyed using topographical stations throughout 2016, 2017, 2018, and 2022. Additionally, a historical methodology was applied to trace the progressive changes in the meandering pattern. Google earth imagery spanning four timeframes (2016, 2017, 2018, and 2022) was also analysed to quantify the change in the morphology of banks and explain the features of the meanders in their natural state before significant human settlement and development occurred (as presented in Table 1).

Table 1: Utilized Google Earth Images for Analyzing spatial and temporal change in meandering patterns

Year	x	Y	Elevation (z)	
			Max	Min
2016	32° 08’ 27.14” N	6° 17’ 43.92” w	869	811
2017	32° 08’ 25.48” N	6° 17’ 39.46” w	872	810
2018	32° 08’ 28.30” N	6° 17’ 47.04” w	892	817
2022	32° 08’ 29.20” N	6° 17’ 43.40” w	893	819

The climatic data of Tizi N’Isli and Tilouguit gauging stations (1980 /2022) were used to determine the hydrological response of the Oued El Abid River. These gauging stations were managed by the Hydraulic Oum Err Bia Agency (HOERBA). Besides, table 2 presents different details of these gauging stations.

Table 2:Detailed of used hydroclimatic data

Station name	Number	x	Y	Z	Serie of data	Mean rainfall
Tizi N’Isli	8500	432300	139600	1595	1975/2019	444.19
Tilouguit	8228	422670	158500	1100	1983-2019	362.13

RESULTS AND DISCUSSION

Hydroclimatic characteristics of the studied period. The climatic context of the studied years can be analyzed and interpreted based on the data presented in table 3. The river’s flow discharge (Q) and the amount of precipitation ® exhibit noticeable variations across the years.

Table 3: Hydro climatic context of the studied years

Studied year	**Q (m^3s^{-1})**	**R (mm^{-1})**
2016	175.13	613.32
2017	92.41	484.65
2018	57.62	338.87
2022	56.78	331.56
MEAN	**95.49**	**442.10**
Max	175.13	613.32
Min	56.78	331.56
SD	55.632	134.167

Source: HOERBA 2022

In 2016, both the flow discharge and precipitation were relatively high, with Q at 175.13 m^3s^{-1} and R at 613.32 mm. In the subsequent years, there was a significant decline in both parameters, particularly in 2017, where Q dropped to 92.41 m^3s^{-1} and R decreased to 484.65 mm^{-1}. This trend of reduced values continued in 2018 and 2022. The average values over the studied years indicate a mean discharge of 95.49 m^3s^{-1} and mean precipitation of 442.10 mm^{-1}. The maximum values occurred in 2016, while the minimum values were recorded in 2022. The standard deviation reveals notable variability, with Q showing a standard deviation of 55.63 m^3s^{-1} and R with a standard deviation of 134.16.

These climatic variations are likely to exert a significant influence on the fluvial dynamics of the studied river. Higher precipitation and flow discharge in 2016 might have led to increased water levels and stronger currents, potentially resulting in greater erosion and sediment transport (Ouakhir *et al*., 2021). The subsequent years' decrease in these parameters could have led to reduced water flow, impacting sediment transport rates, and potentially altering the river's channel morphology. The lower precipitation and discharge levels could contribute to sediment deposition and reduced erosional activity, potentially affecting the meandering patterns and overall river dynamics. The observed fluctuations in the climatic context highlight the importance of considering climatic variables in understanding the changes in fluvial systems over time.

Dynamic and changing of river channel within the studied meander. The climatic context of the studied years, as analyzed in table 3, has implications for the fluvial dynamics in the studied river basin. Table 4 presents the dynamics of erosion process mechanisms observed in the El Abid river meander during the different studied years. Three main erosion processes are observed: fluvial erosion, transport processes and deposition, each expressed as a percentage of the total erosion activity for the respective year. Fluvial erosion shows a gradual decline from 25.30% in 2016 to 18.90% in 2022.

Table 4: Dynamic of erosion process mechanisms occurred in the studied periods

Erosion processes (%)	2016	2017	2018	2022
Fluvial erosion	25.30	25.10	23.40	18.90
Deposition area	62.20	55.80	48.10	68.00
Transport process	12.50	19.10	28.50	13.10
Max	62.20	55.80	48.10	68.00
Min	12.50	19.10	23.40	13.10
SD	25.81	19.69	13.04	30.16

Deposition area, referring to the accumulation of sediment, displays variations, starting at 62.20% in 2016 and declining over the years to 48.10% in 2018, but after registered a substantial increase to 68.00% in 2022.

The transport process, the movement of sediments along the river, exhibits variability, with an initial value of 12.50% in 2016, increasing to 28.50% in 2018, and then decreasing to 13.10% in 2022. The maximum and minimum values for each erosion process are also highlighted, showcasing the range of variation.

Standard deviation (SD) values suggest the degree of variability around the mean, with higher SD indicating greater fluctuations in the data.

Fluvial erosion and deposition area seem to be inversely correlated, with changes in one corresponding to changes in the other. This data implies the dynamic nature of the river's morphology and emphasizes the importance of ongoing monitoring to understand and manage erosion and sedimentation patterns in the El Abid river meander.

Discussion on the factors controlling the morphology change

The box plot (Figure 3) visually represents the process of erosion in the downstream of the El Abid River and its relation to the factors of fluvial erosion, deposition area, and transport process. The data points provide insights into the variability of these parameters. The box plot highlights the central tendencies and dispersion of the dataset. In terms of fluvial erosion, the median value appears to be around 24, indicating a relatively consistent erosion rate. The deposition area shows a wider spread, with the median approximately at 55, revealing variability in the areas where sediment settles. The transport process values, concentrated in the lower quartile, suggest that the majority of the data points experience a lower rate of transport processes. Overall, the box plot visually captures the distribution of erosion, deposition, and transport values, aiding in the understanding of their relationships and the variability within the downstream of the El Abid River.

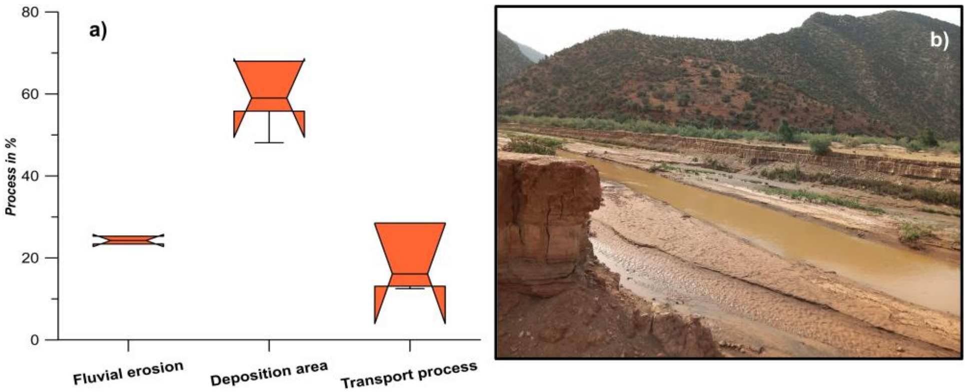

Figure 3. a) Box plot for the importance of erosion processes during the studied period, and b) photos from the field indicating the high significance of deposits and transport dynamics of the studied section

The study of the dynamic changes occurring within El Abid river meanders provides crucial insights into the complex geomorphological processes shaping fluvial landscapes.

The current studied section is subject to continuous alterations driven by various factors such as sediment transport, bank erosion, and flow dynamics what was witness in similar studied regions and comparable researches (Chalise *et al.*, 2019; Tavares *et al*., 2019; Ayt Ougougdal *et al.*, 2020). Such changes impact both the physical characteristics of the channel, including width, depth, and curvature, as well as the ecological and hydrological responses of the surrounding areas (Estrany *et al.*, 2010; Estrany *et al.*, 2011; Estrany and Grimalt, 2014; López-Tarazón and Estrany, 2017).

Studies by authors like Rosgen (1996) and Schumm (1968) underscore the significance of understanding meander dynamics for effective river management and environmental conservation.

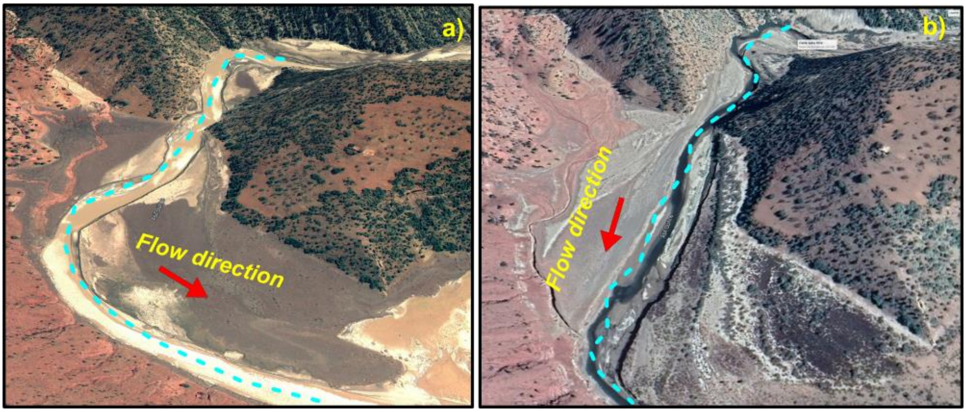

Figure 4. a) Dynamic of erosion process in the studied meander during 2017 and b) 2022. (Google imagery)

By employing techniques such as remote sensing, hydraulic modeling, and geomorphic analysis, researchers can quantify the extent of channel adjustments over time, providing valuable information for sustainable river basin management and mitigation strategies against potential hazards like erosion and flooding. In essence, the study of dynamic changes in river meanders contributes to a deeper comprehension of the intricate interplay between geomorphological processes and the evolving nature of river systems (Figure 4).

Widespread occurrences of meandering channel systems are found in contemporary arid and semi-arid basins, even when vegetation is absent or limited in its ability to stabilize banks and control runoff. Additionally, many of these rivers' traverse regions with varying degrees of vegetation cover, encompassing vegetated and non-vegetated areas (Santos *et al.*, 2019). Surprisingly, these variations in vegetation do not visibly impact the overall arrangement of the channel network, as evidenced by rivers like the Senegal River. The analysis of the El Abid River's meandering behavior demonstrates that the existence of vegetation is not obligatory for the formation of meandering patterns. This stands in contrast to conventional models of rivers before the presence of vegetation, which wrongly assumed that meandering channels rarely developed (Konsoer *et al.*, 2016).

CONCLUSIONS

This research emphasis the erosion dynamics of a meandering section within the downstream part of oued El Abid River and its implications for the morphology change of landscape. The studied section, positioned in the Central High Atlas of Morocco, holds significance as the primary tributary of the Oum-Err-Bia basin. Through extensive field monitoring, the utilization of Geographic Information Systems (GIS), and Google Earth imagery, the investigation started from 2016 to 2022, revealing substantial metamorphosis and ongoing dynamism within the targeted meandering section near the Bin El Ouidane dam. The obtained findings indicate a fluctuating interplay between fluvial erosion, deposition area, and transport processes. However, the erosion mechanisms exhibit changes over time, with fluvial erosion and deposition area demonstrating an inverse correlation. The study underscores the non-linear nature of river systems' responses to modifications in external conditions and highlights the importance of ongoing monitoring for understanding and managing erosion and sedimentation patterns. The research contributes to the broader understanding of meander dynamics, providing insights into geomorphological processes and river system evolution. The findings have implications for effective river management strategies and environmental conservation efforts in regions with similar meandering channel systems.

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THE QUALITATIVE AND QUANTITATIVE ANALYSES OF ECONOMIC, SOCIAL AND ENVIRONMENTAL ROLES OF PERIURBAN AGRICULTURE IN THE CITY OF NIKŠIĆ

SUMMARY

Periurban agriculture is a form of agriculture practiced around urban areas, where it retains both urban and rural characteristics, and which has multiple roles. It has been gaining an increased attention from researchers, practitioners and decision makers, for the potential contribution it can make in the face of current societal challenges. This paper explores the roles of periurban agriculture in Montenegrin context. Through two surveys carried out in the city of Nikšić as a pilot area, economic, social and environmental roles of periurban agriculture have been assessed. The conclusions indicate that periurban agriculture has a significant social role in the target area, unrealised economic potential and an environmental impact that should be better managed. The results are used to argue that periurban agriculture should be mainstreamed into Montenegrin agricultural policy, for its potential to contribute to the quality of life and environmental protection in periurban areas, as well as to food security on the local and national level.

Keywords: periurban agriculture, multifunctional, food security, agricultural policy, Nikšić, Montenegro

INTRODUCTION

Current global crisis, such as the climate change and biodiversity loss, pandemic, wars and conflicts, all emphasize the importance of food security, stability of food supply chains as well as the reduction of the environmental footprint of agricultural production. In this context, the periurban agriculture is gaining an increased interest from both practitioners and researchers around the world.

There is no single unifying and globally accepted definition of periurban agriculture, but all the attempts to define it have several aspects in common: it is

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a human endeavour aimed at producing, processing, marketing and distribution of agricultural food and non-food products from plants and animals within and outside cities (Brown and Carter, 2003). The location of this agricultural production at the fringes of urban areas where it maintains both urban and rural functions is the primary factor that distinguishes it from rural agriculture, with which it can share some common properties (Mngumi, 2020). The resources (material and human) used for periurban agriculture mainly originate in and round a particular urban area, which in turn is usually the main destination of the products and services of periurban agriculture (Addo, 2010). It can play a solely subsistence role, but also be market oriented (Opitz *et al*. 2016).

Periurban agriculture can be a remnant of the previous agricultural activity on the land encroached by the expanding urban area, but increasingly it is a new trend driven by various other socio economic and environmental causes. Some of these key drivers of the rise of periurban agricultural include: the need for achieving food security in times of disrupted food supply chains and rising food prices, increased unemployment rates and the need for new or additional income generation options, exploration of new business opportunities, the urban population's need for recreation, migration of retired people to periurban areas, the pursue of healthier lifestyles by the younger population, and the growing environmental concerns (Dubbeling and de Zeeuw, 2010; Zasada, 2011; Gyasi *et al.* 2014).

This type of agricultural activity is considered to be multifunctional, providing economic, social and environmental functions to the surrounding urban and rural areas (Mngumi, 2020). Multifunctionality is reflected in the following: priurban agriculture provides the agricultural products - mostly perishable foods – like fresh fruits, vegetables, dairy, meat and poultry, but also non-food products, such as fibre, animal feed, medicinal/pharmaceutical and ornamental plants, which can be subsistence oriented, but also be marketed to other urban areas as well as tourism sector. By providing easily accessible, nutritionally adequate, affordable and culturally acceptable food to urban dwellers, periurban agriculture reduces food insecurity and shortens the food supply chains, making them more resilient (Brown and Carter 2003, Lang and Barling 2012, Kortright and Wakefield 2011, Smith *et al.* 2013,Opitz *et al.* 2016). It generates formal and informal employment along the supply chain and can provide new business opportunities.

Periurban agriculture also plays a recreational role – it is itself a leisure activity for urban dwellers (especially retirees), and the maintenance of semi-natural landscapes in periurban areas increases the landscape aesthetics, valued by the urban dwellers (Carrus *et al.* 2015) that use them for recreation. Opportunities for the interaction with the nature and biodiversity that periurban agricultural landscapes offer contribute to mental health and overall wellbeing (Livesley *et al.* 2016). In an increasingly modernised society, periurban areas still preserve pockets of rural lifestyles, maintain connections with local traditions, cultural heritage and the natural environment. With their hedgerows, treelines, ponds, grasslands, periurban agricultural areas provide a patchwork of suitable habitat for biodiversity, including endangered and functionally important species, such as pollinators (Snep *et al*. 2006, Maclagan *et al*. 2018, Pandal *et al*. 2020). Additionally, they ensure the supply of ecosystem services, such as the water infiltration and purification (Haase and Nuissl, 2007), flood prevention (Kenyon *et al.* 2008,Wheater and Evans 2009), local climate moderation (Lamptey *et al.* 2005) and carbon sequestration (Freibauer *et al.* 2004, Hutchinson *et al.* 2007).

Apart from considering the benefits and roles of periurban agriculture, it is important to consider some issues that are inherent in this type of activity. Firstly, although it can be market oriented, the practitioners of periurban agriculture are rarely agricultural professionals and don't always have farming background or experience (Dubbeling *et al.* 2010), which means that they won't necessarily apply the best land management practices, optimise inputs and outputs, or preserve soil, land and water resources. The location of periurban land at the fringes or urban areas means it is exposed to various sources of pollution such as industrial complexes, wastewater discharges, landfills and illegal rubbish dumps, runoff from transport infrastructure and urban surfaces (De Bon *et al.* 2010). These two issues also mean that the products of periurban agriculture do not always meet the quality and safety standards for food items, and contamination of periurban agricultural products with pollutants and biological agents can impact the health of consumers (Bryld 2003, Hamilton *et al.* 2014). Periurban landscapes are under continuous threat from urbanisation (Parsipour *et al.* 2019), and agricultural activity and preservation of natural assets in these areas competes with the more profitable land use forms such as the construction of housing, tourism, transport and other infrastructure.

As far as Montenegro is concerned, it has within the last century transitioned from agrarian society into industrial and service oriented one. However, the role of agriculture remains strong, in economic, social, cultural and environmental respect, and it remains one of the key development sectors, recognised by all the strategic documents. The priority of the agricultural policy is to meet the demands of the national market for agricultural products that can be continuously produced within Montenegro (Strategy for Development of Agriculture and Rural Areas 2015-2020). Also, the national spatial plan stresses the continuing role of agriculture in preservation of cultural landscape as well as sustainable management of natural resources through appropriate land use (Spatial Plan of Montenegro until 2020).

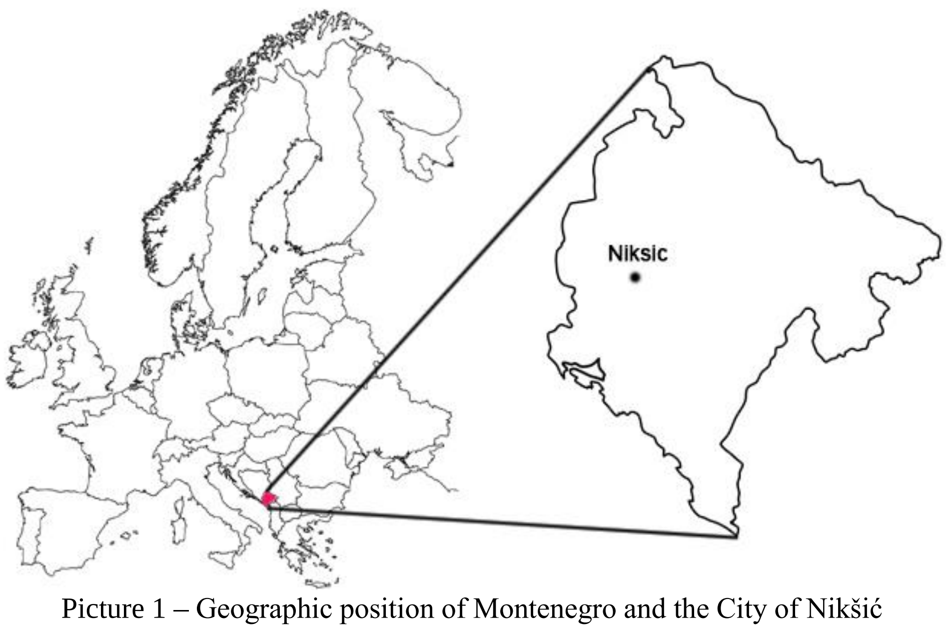
In this respect, periurban agriculture, as a subsector of agriculture, can play an important role, considering its multifunctionality described above. However, it has not been explicitly recognised by the Montenegrin agricultural policy, nor does the policy promote or support the purely subsistence orientation and leisure roles of agriculture, which are typical for periurban agriculture. Yet, as it was seen from the above, these, as well as other functions of periurban agriculture, should be preserved, as they are components of the overall multifunctional role that agriculture plays in Montenegrin context.

Considering the above, this research paper aims to provide the first insights into the roles of periurban agriculture in Montenegrin context.

MATERIAL AND METHODS

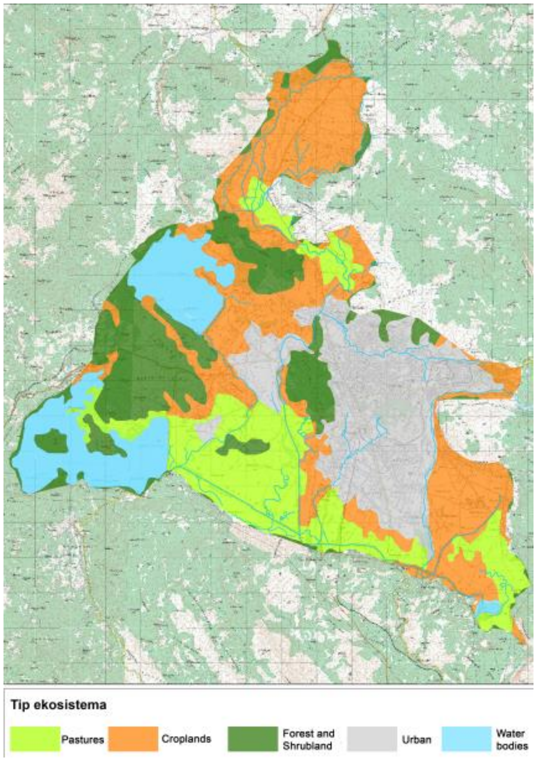
Target area

Nikšić city (central coordinates: 42.775920, 18.922371, picture 1) has been chosen as a pilot site for this research, for several reasons: it is the second largest city in Montenegro, which has in recent years experienced a decline in employment levels and an increase in the number of households and agricultural holdings within the periurban area (Spatial Urbanistic Plan of Municipality of Nikšić until 2020/2025). Agriculture is promoted by municipal development plans, which recognise it as a social buffer, providing additional and frequently the only income for about the third of households in Nikšić municipality (Spatial Urbanistic Plan of Municipality of Nikšić until 2020/2025). Such a setup is common to most other municipalities in Montenegro (Perošević 2020), making Nikšić a suitable pilot area as a model for assessing the roles of periurban agriculture in the wider national context.



Picture 1 – Geographic position of Montenegro and the City of Nikšić

The city is located in the Field of Nikšić, the most compact and continuous agricultural area in the municipality, covering 10578,55ha. Almost half of this area is agricultural land (30.45% of croplands and 18.63% pastures), and almost a third are natural and semi-natural areas (17.9% forests and shrublands, 11.66% water bodies). Urban areas cover 21.36% of the Field of Nikšić (picture 2, source of data – European Union, Copernicus Land Monitoring Service 2018, European Environment Agency). Such structure of land use is indicative of the potentials for periurban agriculture.



Picture 2 – Land use types in the Field of Niksic (source of data: European Union, Copernicus Land Monitoring Service 2018, European Environment Agency)

Surveys

The research has been carried out through two structured surveys with multiple choice and open-ended questions. The first is a household survey, targeting periurban agricultural households, the purpose of which was to determine the role agriculture has for the periurban population, its social, economic and environmental impacts as well as the issues and vulnerabilities of this activity. It contained 50 questions. The number of participants was 48 agricultural households from the Field of Nikšić.

The second was a survey of the general public in the Municipality of Nikšić. The purpose was to elucidate the public perceptions of the economic, social and cultural role of the periurban landscapes and their products in the Field of Nikšić. The survey contained 26 questions. A total of 212 inhabitants participated in this survey.

Both surveys have been conducted online and in person, in the period between February and April 2022. Responses were analysed using SPSS software.

RESULTS AND DISCUSSION

The structure of the periurban agriculture in the Field of Nikšić

From the surveyed agricultural households, more than a half (54.2%) are practicing only plant production, while 41.7% practice mixed production. Only 4.2% households practice animal production only. Dominant products are vegetables (85.4% of participating households) and fruits (52.1% produce woody species apples, pears, walnuts, and 39.6% produce herbaceous and shrubby fruits such as berries and hazelnuts). Over one third (33.3%) of participants have less than 10% of their agricultural land under permanent crops, while little under one third (27.1%) have between 10% and 50%. Every fourth participant has greenhouse production, and the total area under greenhouses among the participants is 1590m^2. Households that raise cattle, sheep and goats mostly use their own pasturelands that are within the Field of Nikšić (64.3%).

Economic roles of the periurban agriculture in the Field of Nikšić

The vast majority of participants – 93.8% - use agricultural products for their own consumption. On the other hand, 27.1% sell their products. Of the producers that sell their products, most are between 20 and 49 years of age (65.1%). No participants older than 65 sell their products. This implies that younger producers (up to 49 years of age) are more oriented towards commercial agricultural production. These participants on average sell around 50% of their products, and the average annual income from these products for the previous year (2021) was 3,900EUR. Most of the sales are through informal channels – 85.7% participants sell their products to individuals within Nikšić (old and new customers, family members, friends, acquaintances), and most of them use internet as a sales channel. As the main barriers to sales, the participants have identified the inadequate state set purchase prices, the lack of organised purchase and the lack of infrastructure such are silos, cold storages and adequate transportation.

The average annual costs of agricultural production for the previous year (2021) were 3,027 EUR.

Almost a third of all participants (29.2%) have plans to expand the agricultural production, and 35.4% are uncertain. It is interesting to note that 35.3% of the participants 20 to 34 years of age do plan to expand the production, while 47.1% are still weighing on this decision. As the most common barriers to the expansion of agricultural production, the participants have identified the lack of agricultural machinery, lack of adequate facilities for production and storage (barns, silos, cold storage) and the lack of adequate financial support instruments.

Only 22.9% of the participants have used some of the incentive measures of the Ministry of Agriculture, Forestry and Water Management or the Municipality of Nikšić. The average amount of subsidies for the previous year (2021) was 4,238EUR. More than two thirds (66.7%) of participants think that the subsidies are adequate. A third (31.6%) of respondents who did not apply for the support measures are not interested; 21.1% stated that they could not fulfil the required conditions and 18.6% did not have the requested information. Also, 10.5% do not know how to access support systems.

Social roles of the periurban agriculture in the Field of Nikšić

For only 14.6% participants, agricultural production is the only or main activity, while for the majority (70.4%) it is a complementary or occasional activity. Agriculture as the only activity is mostly present with the participants between 35 and 49 years of age (42.9%). For 50% of respondents aged 65 and over, it is a supplementary activity. Although it is mostly a complementary or occasional activity, 63.3% participants do not plan to abandon agricultural production, and only 18.4% plan to change the land use on their property.

Other social functions of the periurban areas in the Field of Nikšić were recognised and valued by the inhabitants of Nikšić. According to the survey participants, the main benefits that this area provides to the local community and economy are in the form of provisioning ecosystem services – animal feed (pastures, hay meadows), clean drinking water and local food production, as well as the cultural services in the form of preservation of the local cultural and historic heritage and landscape aesthetics.

Most of the participating inhabitants of Nikšić spend part of their leisure time in the Field of Nikšić (88.7%), and the majority spend between one and four hours per week.

Regarding the origin of their food, for almost half of the participants (49.5%) it is very important to consume locally produced food and they try to maximise their consumption of it. For almost one third of respondents, between 10% and 50% of consumed food is locally produced. The respondents stated that it is necessary to have more domestic food in stores and local restaurants, but also to have appropriate labels and declarations on food, in order to increase the percentage of local food in their consumption.

Almost all respondents (99%) believe that agriculture in Nikšić Field is an important activity for the local economy and development of Nikšić, and they expect that the demand for the locally produced food will rise in the future. On the other hand, over half of the participants (51.4%) are of an opinion that agriculture in the Field of Nikšić is threatened, mostly by the pollution as well as from the lack of public investments.

Environmental roles of the periurban agriculture in the Field of Nikšić

Apart from the cultivated land, 66.7% of responding periurban households also have uncultivated land, that is used as hay meadows or pastures. They have estimated that meadows and pastures comprise between 10% and 50% of their land, which reflects the findings of the national level Agricultural survey from 2011.

They have also estimated the biodiversity values of their land, where the majority of participants identified their property as moderately rich in biodiversity (58.3%), and 22.9% as very rich. 43.8% of responding households are willing to designate a part of their property for biodiversity protection, and 20.8% would be willing to do so with appropriate incentive. This is particularly important, considering that only 6.1% of participants apply some of the nature friendly practices.

The most common land management practices among the participating households are: the use of cattle manure as fertilisers, land tilling every year, and land tilling several times per year. Only 14.3% maintain records of fertiliser use. In terms of irrigation, the majority use water from the public supply network (60.9%), while every fourth depends solely on precipitation (26.1%). One fifth (20.5%) have drip irrigation systems.

The participating households do not have designated waste management systems. Agricultural waste is mostly burned (43.8%) or disposed on part of the property or in local dumpsters (29.2%). The problems with pests and diseases is mostly tackled with the use of artificial pesticides or by manual removal. However, participants do not keep registers of pesticide use, which suggests the need for education and capacity building in respect to pesticide use and their impact on the environment.

The producers believe that climate change significantly affects agriculture, with droughts being the most frequently observed impact.

Discussion

The results of the surveys, have led to the following highlights:

- Periurban agriculture in Nikšić is dominated by the production of fresh plant products (fruits and vegetables), mostly used for own consumption or direct sales locally

- It is a largely informal sector. Agriculture is not a primary source of income for the majority of participants, but rather a complementary or additional activity within the household, and its main goal is not income generation.

- Lack of professional approach is reflected in the fact that most do not make use of the existing subsidy schemes. Furthermore, producers implement agricultural practices that are not always in compliance with the principles of good agricultural practices or environmental concerns

- The distribution chain of products is local and informal, and the local consumers highly value the access to locally produced food, for which there is an increasing demand

- The products are not available in formal distribution, so the access of consumers to the products is limited. This informal nature of sales channels is also related with the somewhat limited consumer trust, as this does not allow for proper labelling of agricultural products, and thus ensuring compliance with the food quality and safety standards.

- The use of resources is not sustainable- especially in relation to water, which is primarily obtained from the public supply network. It is to be expected that the lack of enforcement of good agricultural practices in terms of fertiliser and pesticide use has negative environmental impact.

- Periurban agriculture in Nikšić is vulnerable to climate change impacts, particularly to droughts, and in its current form can cause competition with other forms of water use (especially drinking water).

- The periurban landscapes provide a suite of recreational opportunities that are highly valued by the local urban dwellers

- There is awareness within agricultural producers regarding the need for nature and environmental protection, as well as willingness to contribute to it, but human and financial capacities in this respect are needed.

Research results suggest that the periurban agriculture in Nikšić has an important social function, recognised by the local community, where local food security is more important than economic gain. It also has an economic potential, which, however, can be better realised through adequate support in the form of financing and capacity building. The environmental impact of periurban agriculture in this case can be reduced with capacity building and better enforcement of good agricultural practices and environmental legislation. Finally, this form of agriculture is vulnerable to climate change impacts, to which it doesn't appear to be adequately adapted.

The city of Nikšić stands as a quintessential example of the developmental processes that have characterised Montenegro since the mid XX century (Perošević, 2020), serving as a representative case study for other cities in the country. Expert opinion suggests that analogous circumstances concerning the significance of periurban agriculture and the challenges associated with it are likely to manifest around other urban centers across Montenegro. Therefore, it is important to analyse the periurban agriculture in the wider context of Montenegrin agricultural policy.

Montenegrin agricultural policy is almost exclusively production oriented. Although the policy recognises the multifunctionality of agriculture, it views it as a primarily economic activity associated with a continuous rural area. This is reflected in the design of subsidy schemes, where eligibility criteria such as minimum farm size (in terms of land area and/or number of animals) and farm location are often the limiting factors that small scale periurban producers cannot meet. As such, national and municipal agricultural and developmental policies do not explicitly recognise nor treat periurban agriculture.

For the reasons discussed above, we argue that this form of agriculture should be given more attention in Montenegrin agricultural policy. The first step in this process is to formally recognise it as a legitimate form of land use and mainstream it by providing definitions and designing specific measures that will offer a stimulus for periurban population to continue managing their land in a way that preserves environmental and social benefits in these areas, and not only stimulate the income generation and output maximisation. Periurban agriculture should also play a strategic role in the spatial and development planning processes, because it can enhance the quality of life and preservation of land, biodiversity and cultural heritage in the zones surrounding urban centres. Recognising this form of agriculture will also ensure that the risks associated with it (pollution, land degradation, food safety etc) can be mitigated through enhancement of the relevant legal framework and its enforcement.

An additional rationale for placing focus on periurban agriculture is its potential role as an experimental platform for the implementation of the "smart villages" concept. This innovative approach has gained prominence as a potential contributor to the sustainability and resilience of communities, especially in rural areas (Despotović *et al.* 2020, Garcia Fernandez and Peel, 2023).

Mainstreaming of periurban agriculture into policies and legislation should be accompanied with dedicated capacity building programs. In particular, the periurban farmers need to improve their knowledge and skills in relation to environmental and food safety standards and adaptation to climate change impacts. Advisory and extension services, as well, should have a raised awareness and skills to provide their services in this respect.

CONCLUSIONS

Periurban agriculture provides a suite of benefits to local communities, which can be economic (commercial and non-commercial), cultural and environmental. As such it offers an opportunity to restructure farming beyond pure commodity production. Furthermore, periurban agriculture is viewed as one of the ways to contribute to addressing challenges of food insecurity, urban poverty and adaptation to climate change, and other issues originating from the increasingly urbanised and rapidly changing world. Finally, it should be born in mind that periurban agriculture is an integral component of urban systems, and as the urbanisation continues and the crises intensify, it is to be expected that this form of agricultural production will gain even more importance. Considering all this, as well as the fact that the umbrella objective of Montenegrin agricultural policy is ensuring the food security, we believe that periurban agriculture should be given more attention in the national agricultural policy.

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LICHENS AS THE BIOLOGICAL INDICATORS OF AIR POLLUTION IN THE BIO-MONITORING SYSTEM USED ON ICP SAMPLE PLOTS LEVEL II IN SERBIA

SUMMARY

Lichens, a symbiotic association of fungi and algae, are suitable to serve as a bio-indicator of air pollution due to their ability to absorb toxic materials into their thallus. The photobiont performs photosynthesis; it belongs to blue-green algae, i.e. cyanobacteria and/or green algae; a mycobiont is a fungus from the Ascomycetes, Basidiomycetes or Deuteromycetes group.

Biodiversity Index (LBI) which was used to determine the effect of phytotoxic gases released from pollutants on the diversity of lichens, especially on Level II Sample Plots with conifers. One of the goals of the study is to determine which species of lichen are the most tolerant and the most sensitive to air pollution. This is one of several different methods that have been developed to monitor the environmental quality. One of them, which was used as basis for obtaining the basic statistical parameters, with the subsequently used T-test that confirmed some of the basic initial assumptions, is the significance of sample surface coverage by lichens. The percentages of lichen coverage of the recordings were obtained with basic photo processing programs, most simply viewed in black and white technique.

The results are represented graphically through maps. Different aspects of the current biomonitoring should be compared to the results obtained in practice to date, in order to increase the number of the related studies in the years to come. Lastly, the current studies, progress, and challenges of biomonitoring using lichen as a biological indicator were discussed, and relevant recommendations were formulated.

Keywords: biomonitoring, lichen, air pollution, conifers, LBI, Serbia

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INTRODUCTION

The need for control and monitoring of the air quality ensued from the vital necessity of clean air for survival of the entire living world, where any disruption in the proportion between its basic components directly disrupts the sustainability of the whole ecosystem with all its aerobic denizens.

The goal of control of the air quality and the quantity of its elements is to register and monitor the changes in the amount of these substances in the air, identify the sources and levels of pollution, and assess the degree of congestion at certain locations in order to identify the most critical ones. As some of the cities in the Balkans have lately been ranked high on the ‘most polluted’ list, even in comparison with some of the world’s major metropolises, this type of information has become not only a vital issue of national importance, but also a safety issue that jeopardizes the basic existence itself.

Testing the air quality using lichens as bioindicators is quickly gaining contemporary significance and popularity worldwide (Abas, 2021, then according Ajtic *et al*, 2018; yet it is far less present in Serbia). Lichens are particularly sensitive to increased concentrations of SO_2, which suggests that lichen air-quality index maps may be created once their quality and quantity is registered (Besermenji, 2007).

Further, Bozkurt, 2017; Boonpeng *et al.*, 2018; Diamantopoulus *et al.* 1993; Kunze, 1972; Leblanc and Rao, 1973; Vokou *et al.* 1999 had also made progress with studies still in very early stages and their results certainly an insufficient starting point for drawing any conclusions. Registering the air pollution through use of indicator organisms is highly significant as it represents a relatively simple, quick and inexpensive, but above all harmless and environmentally-friendly way to conduct the monitoring of the quality of the environment. The organisms most frequently used for this purpose include lichens, mosses and vascular plants, where lichens stand out as the most relevant. They feed non-selectively and absorb nutrients exclusively from the air, thus taking in harmful substances along with the nutrients. Since their thallus has no protective surface layer, pollutants readily diffuse into the tissue. There are three basic types of thallus distinguished according to appearance - crusted, leafy and bushy, but their groupings of growth forms are more complicated: fruticose, or flattened; foliose or flat, leaf-like lobes; crustose – crust-like; squamulose – formed of small leaf-like scales crustose; leprous – powdery; gelatinous – jelly-like; filamentous – stringy or like matted hair and busied. Some are completely structureless.

This study aimed to assess the air pollution status in Serbia based on Lichen Another very important property of lichens is that they belong to organisms with continuous vegetative period, which means that they remain active throughout the year. However, not all lichen species are equally sensitive to different levels of air pollution, which provides the opportunity to use them for detecting the presence and activity of pollutants or changes in the air quality.

The notable success of this, provisionally speaking, symbiotic relationship between lichens and conifers (in the case at hand) on our ICP Level II sample plots is directly linked to the balance and parity of metabolisms of these two types of organisms, and may be formed only in a pollution-free environment (first zones of protection of the natural assets, etc.). Such “sterile” environments are extremely difficult to find in the modern world. For this reason, the studies conducted within the framework of the ICP Forests Project, referenced in part in this paper, additionally rely on cross-border pollution, i.e. pollution coming in from distant zones. The effects of pollution on lichens are manifested in a very obvious way: through the decrease of their abundance and coverage, even at lower concentration of pollutants, while they may completely disappear from severely polluted areas, thus creating so-called “lichen deserts”, which are a serious cause for alarm.

Two main biomonitoring techniques using lichen were identified, with varying research scope and types of parameters researched in the last two years. Previous annual studies on ICP Sample Plots Level II in Serbia from the last decade could help compare and analyse not only the methods for gaining insight into the current practices, but also the progress and challenges that may be expected (Conti, 2008).

This is also the fact that we emphasized, which relates to the two currently most used methodologies worldwide for air quality monitoring through lichen diversity. High naturalness, or wilderness, meaning the complete absence of anthropogenic influence, greatly favors the development, abundance, and coverage of lichens, so such surfaces can be called "deserts," but in other extremes – where pollution and human impact are entirely absent – they are, in fact, a positive factor and ensure survival.

MATERIAL AND METHODS

Due to the innovative approach used and presented in this paper (use of the Lichen Biodiversity Index – LBI), it is necessary to provide a brief overview of the approach which has so far yielded notable and acceptable results, but from another aspect in terms of methodology.

The method used to date is the lichen air quality indication method, performed through calculation of the value of IAP (Index of Atmospheric Purity) according to the formula: Eq. (1)

$$IAP = \frac{1}{10} \sum_{n=1}^{30} (Q * f)$$

Where:

IAP–Index of Atmospheric Purity, Q–ecological index of each lichen species, f–coefficient representing the coverage of each registered lichen species on every researched site (expressed in the 1-5 value range).

Interestingly enough, the methodological approach of the IAP method is inversely proportional to the LBI method, not only according to the results expressed numerically, but also in procedure. This is the drawback of the IAP method, over which the LBI method has an advantage because the parameter value (concentration of SO_2) need not be considered. This parameter is obtained through the LBI testing itself, which later on facilitates measurements of SO_2 concentration in places where its elevated value is assumed and marked in color on maps, while further analyses can be requested as needed.

Experiences gained in this paper demonstrate that the results of both methods are partially complementary, i.e., that the old and the new method have both identified the same issue, but in two different ways. Due to the factors that have previously not been considered (presence and distance of pollutants, rather than the substance concentration alone), the new method is now more acceptable as the previous one has sometimes led to confusing guidelines. This has been confirmed by our control measuring stations near or within the urban core, where the concentration of SO_2 is inherently higher. However, this neither indicates nor points to altered biodiversity of the species observed in the study.

The lichen air quality indication method comprises detection, determination of species, collecting and locating lichens, based on which the indication zones of various air quality degrees are formed and presented in maps.

Monitoring of the air pollution in the researched area of Kopaonik and Mokra Gora was tested by means of the Lichen Biodiversity Index (LBI), which was calculated and analyzed through the prevalence of lichens, i.e., their biodiversity was calculated against the presence and proximity of industrial plants. The other 18 control sites were located near Belgrade, in the Arboretum of the Faculty of Forestry, or in the city center, in the urban core. The research was carried out in 2022 and 2023 on 30 measuring stations for sampling (6 so-called measuring stations or sites, with 5 sample plots each). The data on the distribution of lichens were recorded and finally analyzed by means of the T-test. Sampling was done by the transect method, on each of five sites located on the ground or in the tree bark (in all 4 cardinal directions), using a 90x90 cm wooden frame (Figures 1 and 2).

Transect represents a simple method where the abundance of an organism is determined based on smaller samples ("sample areas"). These sample areas can take the form of strips or points of varying widths (areas), and are called transects. Transect methods fall into the category of "intermediate" methods, through which absolute indicators of the size of the observed population can be obtained. However, relative indicators can also be derived, much like the index sought here as a result.

Figure 1. Sampling of lichen species and coverage using a wooden frame (Sample Plot Kopaonik)

Figure 2. Used wooden frame and dimensions

Within each site, lichen species were identified and their abundance was recorded.

In addition, the coverage of the surface beneath the lichen tissue is very important. After capturing and reviewing the images, the percentage of coverage (black color) was digitally calculated, which is possible and accessible in any photo editing software (Figures 3 and 3a).

Figure 3. Example, Original captured photograph and coverage values within the frame, and lichen with natural coloring

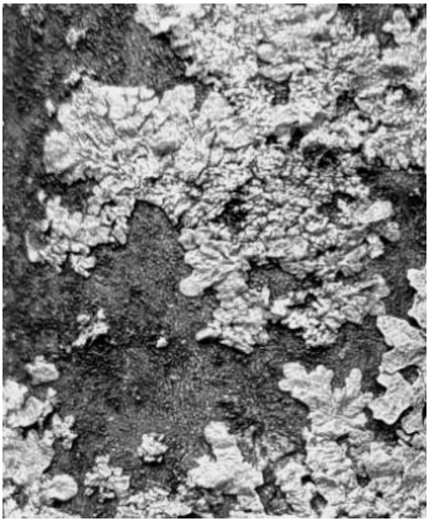


Figure 3a. In the black-and-white (binary) version (technique), the coverage of the lichen on the surface can be easily expressed in %

These values were obtained for all surfaces where measurements were conducted; these are the fundamental data based on which basic statistical values were first obtained (Table 2).

The research on trees was carried out predominantly at breast height - 1.30 m (with the note that the false tissue - plectenchyma of certain lichen species was also found on branches and tree bark, as is the case with the species *Usnea barbata* in spruce stands).

By means of the T-test, a comparison was made to determine whether there was a significant difference between the values obtained from natural diversity on our sample fields (ICP Forest, BIT Level II) and the values obtained around industrial facilities (Table 3).

RESULTS AND DISCUSSION

Clean air and humid environment favor the growth of lichens, while high concentrations of SO_2 confine their growth, which particularly applies to the sensitive type lichens.

The most common lichen species found in the researched area are classified as highly tolerant, such as *Physcia tenella*, *Parmelia sulcata* and *Hiperphiscia adglutinata*, but also as sensitive species, e.g. *Usnea barbata*, *Parmotrema tinctorum*, *Parmotrema praesorediosum*, *Phiscia atrostriata* and *Phiscia pulverulenta*. Knowledge on identification of lichen species and calculation of the LBI results enables determination of the air quality in a certain area, which was the original goal of the research.

Findings are given from each of the measuring stations (total 5 each) located in the vicinity of the above-mentioned plants, due to the different levels of danger to the environment primarily based on the SO_2, emission, as well as from all Level II Sample Plots (Table 1).

Table 1. LBI (diversity) based on sampling according to the transect method, in the vicinity of measuring stations – sample plots 1-6

Sample plots	**LBI results shown on the map**	**LBI in the zone 11-20**	**LBI in the zone 22-30**	**LBI in the zone 33-40**	**Total plots inspected**
1	Sample plot 2 Mokra Gora	4			5
2	Sample plot 2 Kopaonik	2			5
3	Pollutant I – Water bottling plant Kopaonik		17		5
4	Pollutant II – Dairy		13		5
5	Pollutant III – Furniture manufacture “Tomic”		12		5
6	Pollutant IV – Steelworks			35	5
		6	**32**		Total **30**

The obtained results demonstrate that the diversity of lichen species found in a single place enables their use in measuring and quantifying the air pollution levels, both in industrialized areas and in natural reserves. Moreover, within the context of industrial products – air pollutants in cities, it was concluded that motor vehicles have no significant impact on lichen biodiversity, due to the presence of other sources and pollutants that are very easily airborne (Abas and Awang, 2017), which was additionally proven on control samples (Table 2).

Table 2. Basic statistical parameters for measuring stations on 6 sample plots (coverage)

Statistics (in %)	**All sites on 6 habitats (6 sites with 5 sample plots each)**	**Total for all habitats (plus, control)**
Minimum (min)	6%	1%
Maximum (max)	64%	64%
Mean value ($\bar{y}$)	41.12%	29.73%
Standard deviation (σ)	15.6859%	17.5086%
Sample size (n)	30	48

This confirmed the comparison on the potentially significant difference between the values obtained from natural diversity on our sample fields (ICP Forest, BIT Level II) and the values obtained around industrial facilities (Table 3).

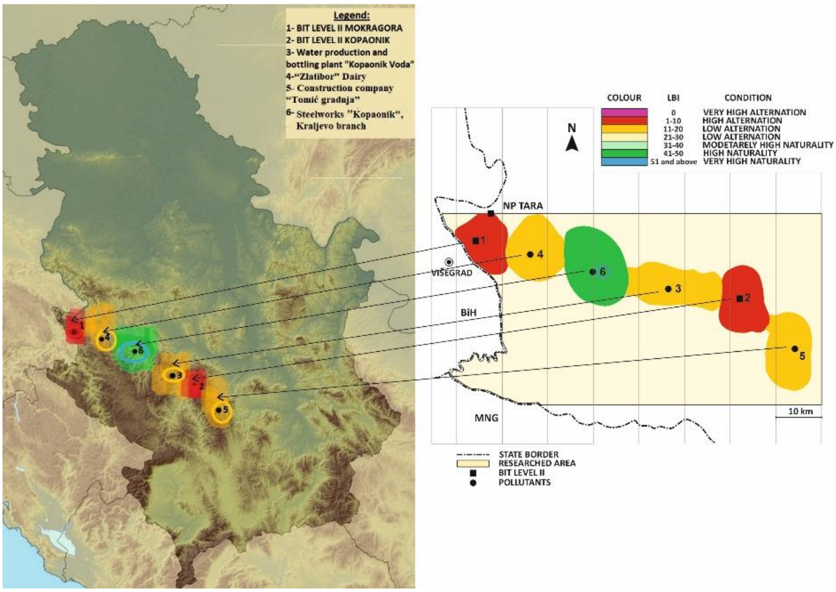
The T-test was calculated on the reliability level $\alpha=0.05$ and the absolute value of t-statistics was calculated at $|-7.992|$, which is associated with t-statistics critical value of 4.0067709. It was therefore concluded that the null hypothesis should be rejected with a 5% risk (Table 3). The alternative H_1 hypothesis was accepted, suggesting that there is a significant statistical difference between mean values expressed in LBI on our samples (there is a possibility that LBI might be higher with the increased presence of pollutants – as presented in maps 1 and 2). Based on the results from 30 control stations (Table 1), high biodiversity was proven on 10 stations, moderate biodiversity on 15 stations, while 5 measuring stations had low biodiversity throughout the researched area.

Statistical analysis through T - test provides a result where P - value between the LBI value and the presence of basic pollutants such as SO_2 amounts to -0.7992 (level of significance $1>r<-1$), and the P value is 0.0009 (level of significance $P<0.05$). This demonstrates that there is an inverted significant relationship (inverse correlation) between the LBI results and the frequency of presence of basic pollutants such as SO_2 (Table 3).

Table 3. Testing of mean values of LBI (coverage) values within 30 (6 study areas X 5 sample plots) (T test)

T test	LBI
Mean value	1.430606061
Variance	9.055871212
Observations (samples pcs.)	30
Pearson correlation	0.000957941
Hypothetical difference between means	0
df (Degrees of freedom)	28
T statistics	-7.992427869
P(T<=t) /One tailed test – distribution	2.0033909
T Critical value for one tailed test	1.693888748
P(T<=t) two tailed	4.0067709
T Critical value for two tailed distribution	2.036933343

Areas with high naturalness – in terms of a consistent lichen response to pollution, or rather – the most commonly used term for the complete absence of primarily anthropogenic influence on the environment where these organisms thrive is lichen desert (0.0%) – were not found at any of the researched sites. Furthermore, naturalness with values exceeding 51 was not observed anywhere (Maps 1 and 2).



Maps 1 and 2. Air pollution zones according to the Lichen Indication Method (LBI) in the researched area in Serbia

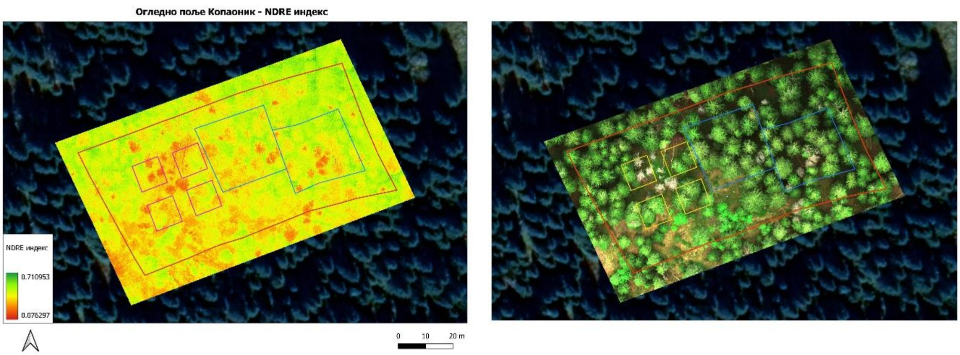
Figure 6. Old-growth forests, or so-called "Virgin" forests, Obed.Bara-Kup.Grede- ravni Srem

Figure 7. *Didymosporina aceris* (Lib.) Mont.)

Declining could be found decline on individual trees or larger forest areas in a locality (Lukyanets *et al.* 2022). Among in general resistant are maples. Only several pests could seriously damage those, such as maple tar spots (*Rhytisma acerinum* Schwein., 1832., Figure 8 and 9) and Maple Leaf Marks (*Didymosporina aceris* (Lib.) Mont.) on *Acer pseudoplatanus* (Figure 7). This is so common in Balkans countries, in the region in general, (Vokou, *et al*. 1999), precisely in or so-called "Virgin" forests (Peruđica, Kupinske Grede), (too many sensitive tree species), and so weak to invasive organisms. This Acer is very common in the floor of the bushes, which represent state of air on level, where actually man is usually co-beneficiary as forest user, which means that, according mentioned pathogens, air has not a positive attribute on this height.

Figure 8 and Figure 9. *Rhytisma acerinum* Schwein., 1832.

The cited research indicates that the study should be expanded through more detailed investigation, e.g. for each individual lichen species, based on their sensitivity to the presence of pollutants (Diamantopoulos *et al.* 1993). This would reveal the current pollution status and open up the possibility of predicting an increasingly urgent need for environmental protection in the future. The NDRE index - Normalized Difference Red Edge index was used, which represents a combination of near-infrared and edge-red light. The index is sensitive to subtle changes, showing aberration (Maps 3 and 4). Its basic techniques, such as content analysis or observation of documents and the use of existing statistics, documents and their secondary analysis, were used to assess the impact of miserable alternation extremes (high and low temperatures) on forest ecosystems and identify possible changes over the years (Češljar *et al.* 2021). This is usually are brought about where changes and improvements of environment, in general are strong and vicious (high and mature old forest in South east Asia, where impact of winds or see currents are strong in forest canopies) (Abas, 2021; Češljar *et al.* 2021).



Maps 3 and 4. The NDRE index - Normalized Difference Red Edge index was used, which represents a combination of near-infrared and edge-red light. The index is sensitive to subtle changes even during late vegetation phases - Kopaonik, Sample Plot Level II.

Mentioned researches indicate that the work should be expanded with a more detailed investigation for each individual species of lichen, based on its sensitivity to the presence of pollutants. Those factors that have occurred in the past to which some other factors have been added (Češljar *et al.* 2021). This would show the current state of pollution and open up the possibility of forecasts of increasingly urgent environmental protection needs in the future (Češljar *et al.* 2021), find decline of individual trees or larger forest areas in a locality (Lukyanets *et al*. 2022.) and all synchronized with previous and so by findings of Ajtic *et al*., 2018 - (RTB Bor in Eastern Serbia); followed also by Bozkurt *et al.* 2017).

CONCLUSIONS

For the development of impact assessment studies during the reconstruction of the existing and construction of new facilities, as well as for the execution of works that can generally affect and even jeopardize the quality of the environment, it is necessary to establish a monitoring system for parameters that reflect that quality, in this case, air quality. This entails general spatial planning and resource utilization measures by introducing measuring stations for air quality control (primarily in areas identified as already threatened or where naturalness is complete, i.e., with no indicators of anthropogenic influence – deserted by lichen), creating an inventory of pollution sources, and maintaining records of the pollution status from these sources. It involves designating and establishing sanitary protection zones around larger industrial and communal facilities, proper maintenance and preservation of as much "naturalness" as possible forest resource management.

By implementing measures of systematic air quality monitoring using the Lichen Biodiversity Index (LBI) method within the investigated area, while simultaneously advocating for reduction in air pollution caused by harmful substances to values below the prescribed limits, as well as taking necessary actions to mitigate their emissions, the necessary changes for returning to a safe and sufficiently healthy environment will occur – a restoration of natural areas with minimal anthropogenic impact. Through intensive monitoring of airborne pollution due to the presence of pollutants in the environment, both natural assets (such as old-growth forests, or so-called "Virgin" forests) and the overall younger but environmental more plastique and more quality will be protected, which would eventually lead to achievement of the ultimate goal - ensuring clean air as a resource essential for human life and health. By comparing and approximating this method, which is more adapted to the countries and forests every day. Eengaging agriculture and fishery, and only conditionally ensure a healthy existence - how is at the crossroads of air and water mass currents, in another sense, from the aspect of economics or management, the situation that is currently unfortunately places our cities and they are often declared as the most polluted "black spots" compared to similar ones in the world and the environment and removed from the list of the most polluted areas. Research like this offers hope and provides a scientific basis to change, improve and bring these and more important existential circumstances under control.

An environment with such a "rating" of air quality (moving active during all the time) would also result in a setting conducive to safe forest management, agricultural activities, and ensuring a healthy existence.

Unfortunately, the current situation places our cities is in a position where they are often labelled as the most polluted, even considered as "black dots" when compared to similar areas globally and in the region. This trial should help to remove them from the list of the most polluted areas. Research like this offers hope and provides a scientific foundation to address and control these crucial aspects of the existence. By continuous monitoring for a longer period of time, it is possible to determine the cause of disease on a certain tree - and bring it into connection with a certain environmental factor.

The prerequisites would also be obtained, which is characterized by such an environment with an air "grade" of sufficient quality to safely manage.

The advantage of this research, in relation to other researches that are performed only after the appearance of decline as the final cause of the influence of this factor, can be seen when these researches, the cause is that moment is very difficult to determine, because it can be initiated by various aspects. Therefore, this type of continuous monitoring can accurately determine the initial stages of the impact of a single, but important in its way, every specific factor.

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MANAGEMENT AND INCOME OF THE ECONOMICALLY MOST IMPORTANT GAME SPECIES OF SERBIA

SUMMARY

The most important hunting game species found in majority of established hunting grounds in the Republic of Serbia are roe deer, wild boar and hare. The objective of this paper is to determine abundance, shooting rate and income generated by managing mentioned game populations in Serbia. On the basis of data of the Republican Bureau of Statistics of Serbia the records on abundance and shooting rate in game species in the 2011-2021 period were collected. The value of shot animals has been determined both on the basis of market pricelist of the Hunting Association of Serbia for the 2021/22 hunting year and according to the structure of animals shot per certain game categories obtained in previous research.

Shooting rate realized in relation to overall roe deer abundance in 2021 was 7.90% with the shooting value reaching 1,106,724 euros. Wild boar abundance index recorded population growth of even 33.53% in 2021 and percentage of population utilization accounted for 63.83% while value of shooting reached 834,509 euros being three times higher than the income of shooting established in 2011. Hare population abundance has recorded a drastic fall and this decreasing trend has been reflected also in a reduced shooting rate thus in 2021 the value of shooting rate of hare was 676,625 euros. The results of the analysis of abundance, shooting rate and trends regarding their values make a basis for indicating a direction of improvement and the measures to enhance the management of these game species populations.

Keywords: roe deer, wild boar, hare, abundance, shooting rate, income

INTRODUCTION

Establishing the abundance of game population is very important for successful management of game population. Determining the number of wild

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game can be either complete if a whole hunting ground is included or partial if it is conducted on a representative sample of hunting ground (Beuković and Popović, 2014). Diversity of habitats and their good quality is essential for the sustainable and effective management of big and small game animals in the hunting grounds (Ozimec *et al.*, 2016). Game population abundance in hunting ground is being determined at the beginning of a hunting year and the counting methods depend on a type of hunting ground and of game species (large or small game). The most significant income from non-wood forest products is fauna of hunting importance (Enescu and Hãlãlișan, 2017). The most representative game species in hunting grounds of Serbia are roe deer, wild boar and hare. (Enescu and Hãlãlișan, 2017).

Roe deer (*Capreolus capreolus* L.), which inhabits almost all of Europe, has an increasing ecological and economic importance and is subject to intensive management, and inhabits over 90% of the total hunting area of Serbia (Kopij, 2023, Popović and Gačić, 2006).

One of the four indigenous species of ruminants distinguished by a wide area of distribution on terrains with different habitat conditions on an overall European continent and in Serbia as well is a wild boar (*Sus scrofa* L.), a very adaptable game species (Novković, 2003; Gačić and Danilović, 2009). In several previous years this species recorded a wide territorial and abundance expansion what resulted in an increased volume of damages on field crops. The shooting of wild boar has been used as one of the ways to reduce the volume of these damages and to alleviate the economic consequences thereof but due to the trophy it is nevertheless very attractive to hunters as well (Scillitani *et al*., 2010; Quirós-Fernández *et al*., 2017).

The hare (*Lepus europaeus* Pall.) is economically one of the most important species of small game in hunting grounds managed by the Hunting Association of Serbia (Popović *et al*., 2012) and the most spread and hunted game in Europe.

The objective of this paper is an economic analysis of a hunting management of the most important game species in Serbia (roe deer, wild boar and hare) through studying both the abundance and shooting rate trends in this game species and a total income generated by shooting.

MATERIAL AND METHODS

The research included hunting activity and economically most representative game species managed by a majority of established hunting grounds according to the Law on Game and Hunting (2010) and the Rule Book on the way of establishing hunting grounds and areas (2018) and the Solution on establishing hunting grounds in hunting areas (2020) in Serbia. Out of a large game a roe deer and wild boar were taken for this analysis while of a small game a native hare species was studied. The records on game abundance and shooting rate in the Republic of Serbia are issued every second year and are taken from the Statistical Bulletin – the Forestry of the Republic of Serbia published by the

Republican Bureau of Statistics of Serbia. A period of observation lasted from 2011 to 2021. The values of shot game were estimated according to a market pricelist of the Hunting Association of Serbia for 2021/22 hunting year https://lss.rs/wp-content/uploads/2022/02/LSS-cenovnik-2021-2022.pdf and are shown in Table 1.

Table 1: Market prices in euros for 2021/22

Game species	**Price (Euro)**
	Shot animals
Trophy roebucks – trophy mass (grams)	
Up to 200,0	50,00
from 200,0 to 249,0	100,00
from 250,0 to 299,0	150,00
from 300,0 to 349,0	200,00
from 350,0 to 399,0	250,00
from 400,0 to 449,0	500,00
from 450,0 to 499,0	800,00
from 500,0 to 549,0	1,200,00
Does	30,00
Fawns	15,00
Trophy wild boar – length of trophy (cm)	
Up to 12,9	50,00
From 13 to 14,9	100,00
from 15 to 17,9	150,00
From 18 to 19,9	200,00
from 20 to 21,9	400,00
from 22 to 23,9	700,00
over 24	1,100,00
Sows	30,00
Piglets and gilts	15,00
Hare	25,00

The shooting structure roebucks and does accounted for 41.33% and in fawns for 17.33%. On the basis of a previous research an average trophy value shown in Table 2 was calculated on the basis of the nine hunting grounds in the territory of Serbia (Barajevska river, Jadar, Takovo, Dubrava, Klisura, Kamenica, Majsinjske mountains, Resava, Krilaš) where 1,184 roe deer trophies were evaluated (Popović *et al.*, 2023) according to the formula of the International Council for Game and Wildlife Conservation CIC (Beuković and Popović, 2014; Ristić and Tiodorović, 2009; Trense *et al.*, 1981; Frković, 1989). An average body mass of roebucks was 20.08 kg (Popović *et al.*, 2017) while of does and fawns it was 17.25 kg and 12.60 kg – respectively (Popović, 1998; Popović *et al.*, 2003).

Table 2: Share of trophies of different mass in total shooting rate of roebucks

Trophy mass (grams)	**Up to 200,0**	**from 200,0 to 249,0**	**from 250,0 to 299,0**	**from 300,0 to 349,0**	**from 350,0 to 399,0**	**from 400,0 to 449,0**	**from 450,0 to 499,0**	**from 500,0 to 549,0**
Average (%)	17.30	15.42	23.56	20.22	13.67	6.83	1.67	1.33

In total shooting rate the structure of individual categories in wild boar was 20.00% for sows and boars and 60.00% for piglets (Novković, 2003). An average body mass of boars and sows was 73 kg (Gajić, 1976) and of piglets and gilts 30 kg (Novaković, 1994) while the share of trophy is displayed in Table 3.

Table 3: Share of individual categories of wild boar trophies in total shooting rate (%)

Trophy length (cm)	**Up to 12,9**	**from13,0 to 14,9**	**from 14,0 to 17,9**	**from 18,0 to 19,9**	**from 20,0 to 21,9**	**from 22,0 to 23,9**	**over 24,00**
Average (%)	30.00	20.00	16.00	14.00	12.00	6.0	2.0

RESULTS AND DISCUSSION

The basis for hunting management is both an accurate estimation of the number of animals and insight into the real conditions that exist in individual game populations (Ćosić *et al*., 2022). The fluctuations in game number depend on a studied game species. On the basis of the Republican Bureau of Statistics the estimations on population number of the most important game in Serbia (2011-2021) indicate an increasing trend roe deer with the difference in 2021 being 27,489 roe deer and a growing rate of 4.29% compared with 2011. An average shooting rate realized in relation to total number of roe deer ranged between 6.84% and 7.95% (Table 4).

The abundance of wild boar in Serbia shows that the wild boar population is increasing along with the volume of shooting. Number of wild boar in Serbia in 2021 was 23,856 individuals the rate of increase in number of population being 5.95% compared with 2011. The shooting rate in 2021 increased by 25.14% compared to 2011. A utilization percentage of wild boar population in relation to number of wild boar was 63.83% in 2021. The abundance index of wild boar in the last year of research compared to basic 2011 shows the increase in the population by even 33.53%. The results of the other authors also indicate the increasing abundance in wild boar for which one of the reasons could be a discrepancy between a planned and realized shooting (Djan *et al*., 2013; Lavadinović *et al*., 2020). The research in the 1980-2000 period showed that the trend of shooting rate in wild boar is parabolic with shooting rate decreasing by 1% annually (Ranković and Popović, 2002).

The number of hare varied in the interval of 430 thousand to 501 thousand in the period from 2011 to 2021 and the abundance index of hare population in the last year of observation, i.e. 2021, recorded the fall in population by about 5% compared with the basic 2011. In addition, there occurred the change in hare shooting rate where a falling trend was noticed along with a negative growth rate of 14.61%. Hare population is decreasing not only in our region but also throughout whole Europe. Many authors suggest that the decrease in hare number is caused by human activities (use of arable land, traffic, hunting), climate (precipitations and temperature) and/or diseases (Popović *et al*., 2014; Beuković *et al*., 2013). According to the Law on Game and Hunting (2010) the wild game can be introduced into a hunting ground only if by their introducing the balance between biological equilibrium and biological diversity has not been disturbed. Introduction of hares into the hunting grounds of Serbia were mostly conducted by catching the hare in Vojvodina hunting grounds and discharging them in the hunting grounds of Central Serbia. However, as their number decreased in the last several years there is no user of hunting ground who will catch and sell hare while hare breeding on farms in Serbia is practically non-existent.

Table 4: Abundance and shooting rate of roe deer, wild boar and hare in the Republic of Serbia

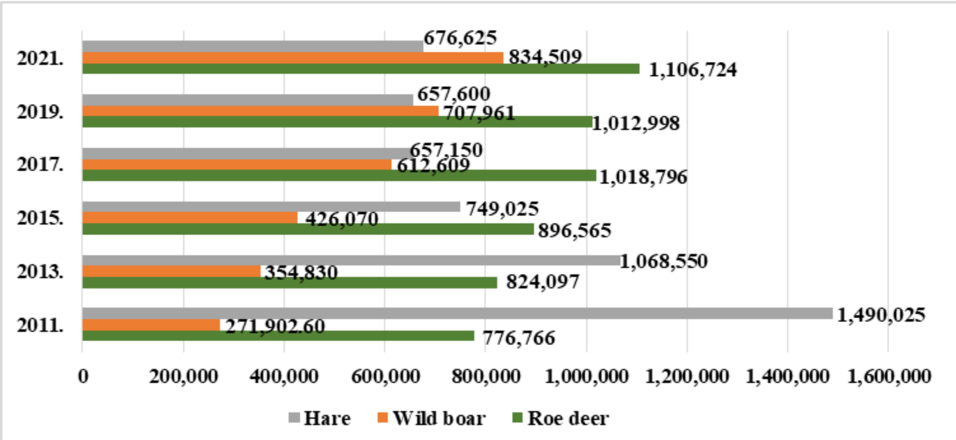
Year	Roe deer				Wild boar				Hare			
	Number state	Shooting rate	% utilisation	Number index	Number state	Shooting rate	% utilisation	Number index	Number state	Shooting rate	% utilisation	Number index
2011.	117.502	8.039	6,84	100,00	17.865	4.962	27,77	100,00	501.456	59.601	11,89	100,00
2013.	120.095	8.529	7,10	102,21	23.163	6.475	27,95	129,66	486.105	42.742	8,79	96,94
2015.	127.853	9.279	7,26	108,81	21.288	7.775	36,52	119,16	454.887	29.961	6,59	90,71
2017.	132.642	10.544	7,95	112,88	23.701	11.179	47,17	132,67	430.690	26.286	6,10	85,89
2019.	141.602	10.484	7,40	120,51	25.309	12.919	51,05	141,67	447.303	26.304	5,88	89,20
2021.	144.991	11.454	7,90	123,39	23.856	15.228	63,83	133,53	478.111	27.065	5,66	95,34

Source: Statistical Bulletin – the Forestry in the Republic of Serbia, RBS and the author`s calculation

Analyzing the income obtained by shooting roe deer, wild boar and hare in the period from 2011 to 2021 great differences can be noticed (graph 1). The roe deer generated income shows an increasing trend being 1,106,724 euros in 2021 what resulted in higher shooting rate and share of male deer trophies in shooting rate quotas. An early shooting of male deer is forbidden (Urošević *et al.*, 2017) and a research conducted in our country (Urošević *et al.*, 2013) confirmed that male deer were shot before they finished their body development and therefore trophies could not attain their maximum value. Trophy mass increases with the age of an individual and the highest quality antlers were found in the individuals aged six and seven when shooting should actually take place (Popović *et al.*, 2020).

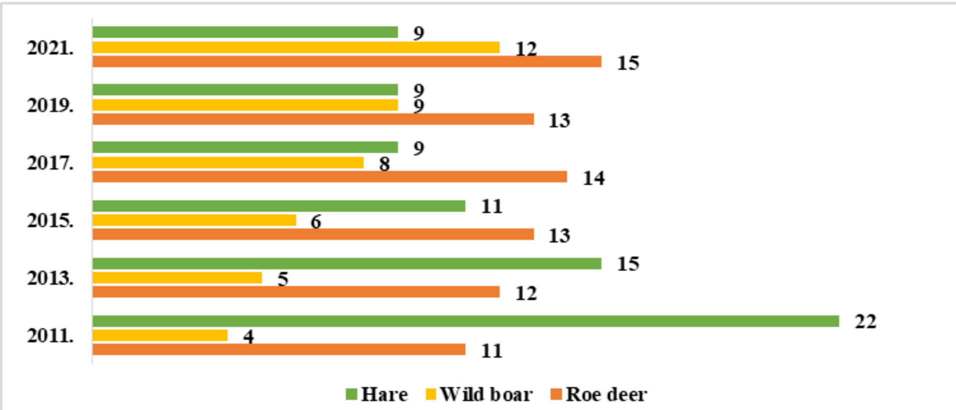
In addition, the income generated by shooting wild boar registered the increase in each studied year and in 2021 the value of shooting was 834,509 euros what was three times higher compared with the profit of shooting determined in 2011.

When it comes to total hare shooting income as an economically the most important species of small game its number in the last years in Serbia decreased considerably what resulted in decreased shooting incomes as well. The shooting value in the observed period decreased from 1,490,025 euros to 676,625 euros what is a decrease of 45.41 %.



Graph 1. Total income of shooting the roe deer, wild boar and hare in the Republic of Serbia in euros in the 2011-2021 period

The income generated on total 100 ha of hunting ground in the Republic of Serbia was observed (graph 2) and in 2021 the realized income for roe deer was 15 euros, for wild boar 12 euros and for hare 9 euros.



Graph 2. The income on 100 ha generated by shooting the roe deer, wild boar and hare in the Republic of Serbia in euros in the 2011-2021 period

The improvement of the economic results of managing the large and small game populations can be accomplished by improving the age structure of roe deer population, by increasing the share of male deer with trophy mass of over 450 grams, decreasing the losses and increasing the real weight growth in some game species in acceptable biological limits (Popović and Popović, 2021).

CONCLUSIONS

By the analysis of abundance, shooting rate and income in hunting grounds of Serbia in the three most important hunting species (roe deer, wild boar and hare) it was determined that the roe deer is the most distributed species of large game and the most important game of high hunting from the economic aspect followed by wild boar while in hare the trend of decrease of abundance and shooting has been observed entailing a decrease in the shooting value as well. The results of abundance, shooting rate and fluctuations in their values make the basis for indicating the directions of improving the management of populations of these game species. In roe deer and wild boar it seems necessary to increase the percentage of utilization (shooting) of population, to make improvements in age structure, reduce losses and increase a real growth in acceptable biological limits. In hare it is necessary to work on stopping a negative rate of population growth by all the management measures and in the first place to determine a possibility of utilizing the population in a given year on the basis of spring abundance and a population real growth.

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ENVIRONMENT AND DIGESTATE AFFECT ON THE OATS QUALITY AND YIELD PARAMETERS

SUMMARY

Oats are of great economic importance thanks to the high nutritional value of the grain. In this study, the productivity of oats was analyzed in two varieties: control (variants without digestate) and in the variant with digestate, during 2021-2022. The following parameters were tested: plant height, number of grains per panicle, grain yield per hectare and protein content. Highly significant positive correlation coefficients were found between grain yield and number of grains per plants (r=0.68**) and grain yield and plant height (r=0.59*). The results showed that year and digestate had no statistically significant effect on oat grain yield. The obtained values for grain yield were statistically significantly higher in the variant with digestate compared to the control variant. The digestate had a significant effect on the increase of oat yield and yield parameters, therefore its application in the oat crop is recommended.

Keywords: oats, year, digestate, yield components, protein content

INTRODUCTION

Oats (*Avena sativa* L.) is a real cereal that is of great economic importance due to the grain that has a high nutritional value, which is why it has been declared a functional food. Oat grain is desirable in the diet because it is responsible for numerous health benefits (Burić *et al.*, 2023). In human nutrition, peeled oat grain is used in the form of oatmeal, semolina and oat flour, which is

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mixed with wheat to make bread and other bakery products (Figure 1a-c). Oat grain has 8.18% of digestible proteins and it has a higher nutritional value than maize (Glamočlija *et al.,* 2015; Rajičić and Terzić, 2022; Burić *et al.,* 2023; Popović *et al.,* 2023). In Serbia, more it is grown as a forage plant in mixtures with legumes, and less for grain. In the world, the area under oats is decreasing at the expense of more productive grains, and according to FAO data, in 2022, it was grown on 9,562,497 ha. The average grain yield was 2,360 kg ha^{-1}, and the total production was 22,571,618 tons. The largest areas under oats by continent were in Europe (5,390,227 ha or 56.37%) and America (2,387,873 ha or 24.97%). The highest average grain yield of 2,526 kg ha^{-1} and a total production of 13,614,876 tons were recorded In Europe. In Serbia, oats was grown on 14,503 on which 44,176 tons of oat grains were produced. The average grain yields of 3,046 kg ha^{-1} are 20.58% above the average of European countries, i.e. about 30% higher than the world's average yields (Popović *et al*., 2023).

These types of soils are characterized by different properties (chemical, physical, and physical-mechanical, productive). The agrotechnical and meliorative measures are determined based on the properties of the various soil types in the target of improving their productive capacity and agricultural production (Markoski *et al*., 2018; 2021a; 2021b; Boitumelo Mohlala *et al*., 2022; Dugalić *et al*., 2022; Stevanović *et al.*, 2023; Sekulić *et al.*, 2023). For most types of soil in Serbia conditions, to achieve high yield and good grain quality, on average, 60-90 kg ha^{-1} of N, 60-90 kg ha^{-1} of P_2O_5 and 40-60 kg ha^{-1} of K_2O pure nutrients should be applied. Phosphorus and potassium fertilizers are introduced in winter oats 50% in the basic tillage and 50% before sowing, while in spring oats all phosphorus and potassium quantities are introduced in autumn under basic tillage. In more humid regions, the amount of nitrogen for fertilization is added early in the spring during intense tillering (the first fertilization with half of the anticipated amount) and the second fertilization at the beginning of stem elongation with the remaining amount of nitrogen. In the case of spring oats in arid regions, the entire amount of nitrogen is given before basic treatment or pre-sowing preparation, i.e. without top dressing (Rajičić *et al.,* 2020; 2021; Popović *et al*., 2023). The aim of this study was to investigate the effect of digestate on grain yield, productivity parameters and protein content of oat grain, over a two-year period.

MATERIAL AND METHODS

Study area

Experiments with spring oat variety NS Dunav carried out in Kovin, Pančevo municipality during 2021 and 2022, on chernozem soil in three repetitions. The oats pre-crop was soybean. For trial, an elementary plot of 25 m^2 was sown. Soil cultivation was carried out according to the varietal standard growing technology for the tested spring oat variety. Digestate was used as supplement. The experiment was performed in two variants: 1) control variant without digestate and 2) variant with digestate. The entire amount of digestate is given during the pre-sowing preparation. Sowing was done in mid-February, Harvesting was done in early August with a harvester for experiments. The following parameters were analyzed: plant height (cm), number of grains per panicle, grain yield per hectare (t ha^{-1}) and protein content (%). The yield was measured after harvest and recalculated to 14% moisture.

After harvesting, oat grains are stored in silos and dried to 14-15% humidity. Oat grain with a higher percentage of water after harvest is dried in dryers or directly poured into a silo that has an innovative mixing propeller that works with the help of SMART-THINGS, i.e. with the help of sensors as shown in Figures 1a and 1b. The sensors monitor the humidity of the oat grains, report to the central sensor that directly with the help of the aggregate automatically blows moist or dry air, depending on the need in order to maintain optimal conditions for storing oat seeds (Popović *et al.*, 2023).

a) Oats Panicle

b) Oats grain

c)Storage oats silo

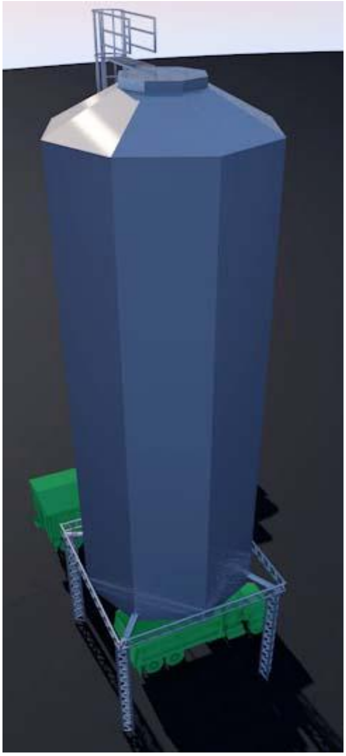

Figure 1. Oats panicle (a.), grain (b.), Storage silo (c.), Designed by (Ristić & Popović, 2023)

Meteorological Conditions

The meteorological conditions, monthly precipitation and air temperatures for 2021 and 2022 during the trial were taken from the Hydro-meteorological service of Republic of Serbia, Pancevo (Table 1).

Table 1. Average monthly temperatures and total precipitation for the oats vegetation period in 2021 and 2022, in Pančevo

Parameter	2021	2022
Average Temperature (°C)	19.25	19.03
Total Precipitation (mm)	328.4	351.4

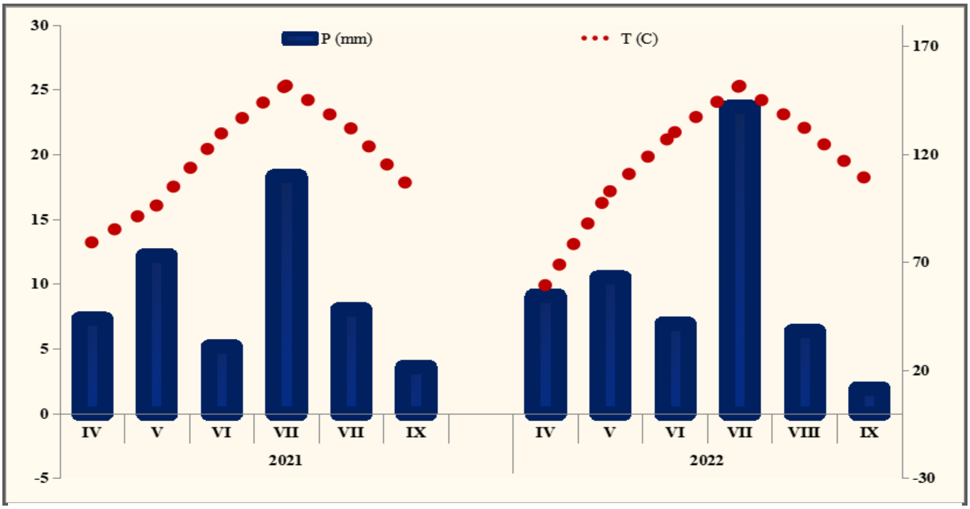

Figure 1. Average monthly temperatures and total precipitation in 2021 and 2022

Precipitation have a decisive influence on the yield components (Howarth *et al*., 2021; Lakić *et al*., 2021; 2022a; 2022b; Ljubičić *et al*., 2021; 2023; Nikolić *et al*., 2022; Dražić *et al*., 2022; Rakaščan *et al*., 2021; Dimitrijević *et al*., 2022; Filipović *et al*., 2022; Milunović *et al*., 2022; Popović *et al*., 2011; 2020a-b; 2022; Kosev *et al*., 2022). Average temperatures in the growing season were 19.25°C in 2021. and 19.03°C in 2022., while total precipitation in 2022 was 351.4 mm and it was higher by 23 mm then in 2021, figure 1.

RESULTS AND DISCUSSION

Productive characteristics and protein content of oat in Serbia

The average value for the height of the plants was 138.5 cm. The values for plant height varied from 138.5 cm in the control variant to 159.20 cm in the variant with digestate. The variant and the Y×V interaction had a statistically significant effect on plant height values. The year had no statistical significance for the investigated factor, tables 2 and 3, figure 2a.

The average value for the parameter - number of grains per panicle - was 48.50. The values of the number of grains per panicle varied from 44.33 in the control variant to 52.66 in the variant with digestate. The variant and the interaction of the examined factors V×Y was statistically significant for the examined factor (tables 2 and 4, figure 2b). A more favorable year for the number of grains per panicle was 2022 compared to 2021. In 2022, the number of grains per panicle increased by 4.5%.

Table 2. Parameters of oat productivity in Pančevo, 2021 and 2022

Parameters	Variant	2021.	2022	Average	IV*
Plant height, cm	Kontrola	138.3±1.87	138.7±1.20	138.5±1.41	0.40
	Digestat	161.0±0.72	157.3±0.15	159.2±0.51	3.70
	Average	**149.7±1.77**	**148.0±1.28**	**148.8±1.48**	**1.70**
Number of grains per panicle	Kontrola	41.33±6.11	47.33±5.51	44.33±6.15	6.00
	Digestat	53.66±2.31	51.66±3.06	52.66±2.66	2.00
	Average	**47.50±7.92**	**49.50±4.64**	**48.50±6.27**	**2.00**
Grain yield, t ha^{-1}	Kontrola	2.73±0.23	3.28±3.29	3,01±0.41	0.55
	Digestat	3.69±0.09	3.81±0.19	3.75±0.15	0.12
	Average	**3.21±0.55**	**3.55±0.38**	**3.38±0.48**	**0.34**
Protein content, %	Kontrola	7.00±0.72	5.90±0.26	6.45±0.77	1.10
	Digestat	7.76±0.73	6.83±0.68	7.30±0.81	0.93
	Average	**7.38±0.77**	**6.37±0.68**	**6.87±0.88**	**1.01**

**IV- Interval of variation*

LSD	Plant height		Number of grain pp		Grain yield		Protein content	
	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1
Year	1.559	2.269	6.040*	8.787	0,316	0.461	0.835	1.216
Variant	1.560	2.269	6.041*	8.789	0.317	0.461	0.836	1.216
YxV	2.206	3.209	8.542*	12.428	0.448	0.652	1.182	1.718

Table 3. Anova for plant height

Parameter	SS	Degr. of Fr.	MS	F	p
Intercept	265816.3	1	265816.3	193673.1	0.000000
Year	8.3	1	8.3	0.061	0.811572
Variant	1281.3	1	1281.3	9.336*	0.015691
Y x V	12.0	1	12.0	0.087*	0.774998
Error	1098.0	8	137.3		

Table 4. Anova for the number of grains per plant

Parameter	SS	Degr. of Freedom	MS	F	p
Intercept	28227.00	1	28227.00	1371.352	0.000000
Year	12.00	1	12.00	0.583	0.467058
Variant	208.33	1	208.33	10.121*	0.012970
Y x V	48.00	1	48.00	2.332*	0.165258
Error	164.67	8	20.58		

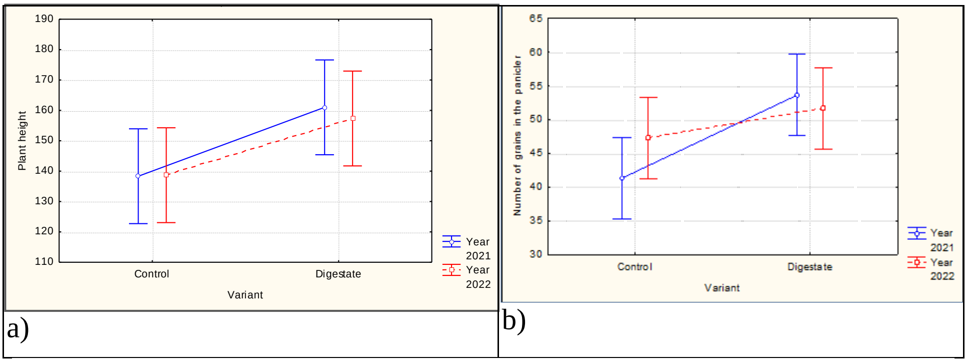

Figure 2. Interaction Y×V for planth height (a) and number grain per panicle (b)

The average value for grain yield was 3.38 t ha^{-1} and varied from 3.01 t ha^{-1} in the control variant to 3.75 t ha^{-1} in the variant with digestate. Year, variant and interaction of the examined factors were statistically significant for the examined factor (tables 2 and 5, figure 3a). A more favorable year for grain yield was 2022 compared to 2021. In 2022, a statistically significantly higher grain yield was achieved, by 340 kg ha^{-1}, i.e. by 11.33% (tables 2 and 5).

Tabela 5. Anova for grain yield

Parameter	SS	Degr. of Free.	MS	F	p
Intercept	137.2280	1	137.2280	2422.384	0.000000
Year*	0.3333	1	0.3333	5.884	0.041479
Variant*	1.6576	1	1.6576	29.261	0.000639
Y x V*	0.1452	1	0.1452	2.563	0.148051
Error	0.4532	8	0.0566		

The average value for protein content was 6.87% and varied from 6.45% in the control variant to 7.30% in the variant with digestate. The year, variant and interaction of the examined factors were statistically significant for the examined factor (table 2, figure 3b). The more favorable year for protein content was 2021 compared to 2022. In 2021, statistically significantly higher values were achieved for protein content, namely for 15, 85% (tables 2 and 6, figure 3b).

Tabela 6. Anova for oat grain protein content

Parameter	SS	Degr. of Freedom	MS	F	p
Intercept	567.1875	1	567.1875	1438.953	0.000000
Year*	3.1008	1	3.1008	7.867	0.023027
Variant*	2.1675	1	2.1675	5.499	0.047048
Y x V*	0.0208	1	0.0208	0.053	0.823939
Error	3.1533	8	0.3942		

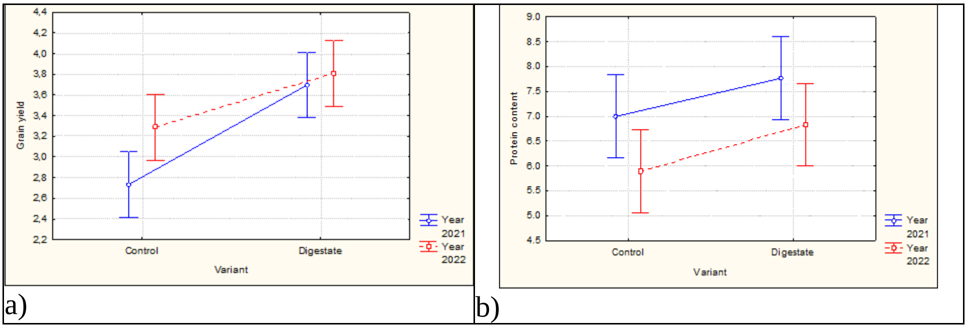

Figure 3. Interaction Y×V for grain yield (a) and protein content of oats grain (b)

Jordanovska *et al*. (2018) points out that the protein content of oat grains varied from 12-15%, fat content from 4-6.5% and crude fiber content from 12.2-12.5%.

Correlation analysis of the studied oat traits

Correlation coefficients based on all tested traits during 2021-2022 had positive values (Table 7). Genotypic correlation coefficients provide a measure of the genetic association among characters and give an indication of characters that could be useful so as to identify more important ones for a particular selection programme. Correlation studies were conducted to study the degree of interrelationship between grain yield and their major traits.

Table 7. Correlations between the analyzed traits

Traits	PH	NoG	GY	PC	Temp.	Prec.
Plant height - PH	1.00	0.16 ns	0.59*	0.24 ns	0.54*	0.76**
Number grain per panicle - NoG	0.16 ns	1.00	0.68*	0.14 ns	0.83*	0.66*
Grain yield-GY	0.59*	0.68*	1.00	0.04	0.76*	0.80**
Protein content-PC	0.24 ns	0.11 ns	0.04 ns	1.00	0.41 ns	0.52*
ns - non significant; * significant at 0.05; **significant at 0.01						

Highly significant positive correlation coefficients were found between grain yield and number grains per plants (r=0.68**), grain yield and precipitaion (r=0.76*), grain yields and temperature (r=0.76*) and grain yield and planth height (r=0.59*). Significant positive correlations were found between number of grains in panicle and precipitation (r=0.66*) and planth height and temperature (r=0.59*).

Protein content did not have a significant correlation with grain yield (Table 7). A strong positive correlation between small yields and grain weight has been found by many researchers (Terzic *et al*., 2018), medium (Đekić *et al*., 2012; 2014; 2018; Güngör *et al*., 2017), while weak positive dependence has been identified by Rajičić *et al.* (2020). Grain yield plant^{-1} exhibited significant and positive correlation coefficients with spike-lets panicle^{-1}, 1000 seed weight (g) but negative non-significant correlation with protein content (Ahmad *et al*., 2013). These results will be beneficial in devising a selection scheme for identifying best genotypes possessing higher forage yield, grain yield and better quality.

CONCLUSIONS

Oat grain is used for nutrition as a grain but also for non-food products and due to its unique grain quality, it is known as a functional food. The results showed that year and digestate had statistically significant effect on oat grain yield. Statistically significantly higher grain yield values were achieved in the variant with digestate compared to the control variant, which is why its application in the oat crop is justified. Highly significant positive correlation coefficients were found between grain yield and number of grains per plants (r=0.68**), grain yield and precipitation (r=0.76*), grain yields and temperature (r=0.76*) and grain yield and plant height (r=0.59*).

Big progress has already been achieved in breeding oats. Breeders of oats need to make available to the market higher yielding and higher grain quality cultivars. Therefore, it is necessary to optimize the strategies of selection of superior oats genotypes.

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Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used etc.

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Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

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The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

- REFERENCES (LITERATURE)

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