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## CONTENT

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# STUDY OF MONOCULTURE AND MIXED GRASS AND LEGUME FORAGE CROPS UNDER MOUNTAIN CONDITIONS

## SUMMARY

The aim of the study was to determine the productivity, growth and development of perennial grass forage crop species (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown in sole crops and binary mixtures with *Trifolium pratense* L., under mountain conditions.

It was found that in the sole grasses, the crops of *Phleum pratense* L. were proven to have the highest yield of fresh (2279.2 kg/da) and dry mass (929.3 kg/da). The excess in the values of the indicators was up to 23.8% and 29.0%, respectively. The lowest productivity was recorded in monoculture crops of *Lolium perenne* L.

In the two-component mixtures, the grasses of *Phleum pratense* L. with *Trifolium pratense* L. (2168.7 kg/da) and *Dactylis glomerata* L. with *Trifolium pratense* L. (722.2 kg/da) significantly exceeded the yield of fresh and dry mass compared to the mixture of *Lolium perenne* L. with *Trifolium pratense* L. by 21.2% and 26.5%, respectively.

In the conditions of the Middle Balkan Mountains, the mixture with the most optimal evenness in the height of the grass and legume component is the binary mixture of *Festuca rubra* L. + *Trifolium pratense* L. With the greatest difference in the mean values of the trait is the mixture *Phleum pratense* L. + *Trifolium pratense* L. On average over the period, the stem height of the forage grasses species in the stand-alone mixtures varied from 32.7 cm to 99.2 cm, and in the two-component mixtures from 35.4 cm to 101.9 cm.

**Keywords**: yield of fresh and dry mass, grass-legume mixtures

## INTRODUCTION

In the mountain and foothill conditions of the Middle Balkan Mountains (Bulgaria), the composition of plant associations in natural and sown grasslands is closely related to the biological characteristic of species, their plasticity and stability (Georgieva *et al.*, 2018).

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Representatives of the family *Poaceae* have a dominant influence in the formation of the grasslands type. They are a reliable source of biomass for animals. They are distinguished by high resistance to adverse environmental conditions and undemanding to soil and climatic factors (Landi *et al.,* 2017; Indu *et al.,* 2023; Kociecka *et al.,* 2023). They grow well both in low-lying areas where soils are nutrient-rich and in high-altitude areas on poor and poorly productive land (Kostov and Pavlov, 1999; Valdez and Dumansi, 2020; Kurhak *et al.,* 2021). The valuable forage qualities of meadow grasses (longevity, high productivity with relatively low cost, high carbohydrate and energy content) and the specific features related to the growth and development of the species significantly influence their selection (Lüscher *et al*., 2014).

In the context of sustainable agriculture, the selection of perennial grasses and legumes of high quality and nutritional value has a significant impact in determining the productivity and quality of forage mass, as well as in creating a stable forage base for ruminant livestock production. Their ability to combine is an important factor in maintaining dynamic stability in the herbage stand and securing forage throughout the grazing season (Rolando *et al*., 2018; Vasileva and Enchev, 2018; Vanek *et al.,* 2020).

Compared to monoculture crops, mixtures of perennial grass and legume are also distinguished by higher productivity, ecological plasticity and durability (Helgadottir *et al.,* 2018; Meza *et al.,* 2022). The optimal association of certain species of perennial forage grasses points out the advantages of mixed grasses regarding better utilization of natural resources, individual plant growth and development (Dhakal and Islam, 2018; Tahir *et al.,* 2022).

Considering the upland soil and climatic conditions, successful establishment of sown grasses require legumes to have good ecological adaptation. *Trifolium pratense* L. has been identified as such a species in a number of studies for the area (Naydenova *et al.,* 2010; Naydenova and Bozhanska, 2014; Mihovsky and Naydenova, 2017; Petkova *et al.,* 2023). This species is also a valuable component in the composition of natural and sown grasslands, with grazing and hay use in many areas of Europe (Herrmann *et al.,* 2008; Drobna, 2009). It shows good adaptability to soil type, is highly productive and is successfully incorporated into mixed grass stands. Its above-ground mass is tender and readily accepted by animals (Nedělník *et al.,* 2016).

The aim of the study was to determine the productivity, growth and development of monoculture crops of meadow grasses and their mixtures with *Trifolium pratense* L., under the conditions of the Middle Balkan Mountains.

## MATERIAL AND METHODS

The experiment was conducted in the period 2020-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria), on light gray, pseudopodzolic soils with pH=4.2-5.5 (Penkov, 1988). The objective of the study refers to four perennial species of grass forage (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown as monoculture (100%) and in mixtures with red clover (*Trifolium pratense* L.) in a ratio of 50%:50%, under nonirrigated conditions.

**The experimental variants included:**

Monoculture grass stands

**1.** *Festuca rubra* L. (FR)
**2.** *Lolium perenne* L. (LP)
**3.** *Dactylis glomerata* L. (DG)
**4.** *Phleum pratense* L. (PP)

Two-component mixtures

**5.** *Festuca rubra* L. + *Trifolium pratense* L. (FR+TrP)
**6.** *Lolium perenne* L. + *Trifolium pratense* L. (LP+TrP)
**7.** *Phleum pratense* L. + *Trifolium pratense* L. (PP+TrP)
**8.** *Dactylis glomerata* L. + *Trifolium pratense* L. (DG+TrP)

Sowing was done manually, scattered and the areas were rolled to ensure better contact of the seeds with the soil. The sowing rates of the studied forage species were calculated based on 100% seed germination. The plot size was 5 $m^2$, laid out in 4 replications. Once a year (the last ten days of March - 20-30.03.) mineral fertilizing was applied. The monoculture crops were treated with $N_{12}$ and the mixed grasslands were with a combined fertilizer of $N_{12}$ and $P_8$.

The grasslands were mowed at the beginning of the tasseling/ear formation period for grasses and the bud-formation period/blossoming period for legumes. The weed control during the vegetation was mechanical, intending to not allow additional chemical intervention on the plants.

The following indicators were reported and analysed:

- Meteorological observations - annual and vegetation mean temperatures (ºC) and rainfall totals (mm);

- Fresh and dry mass yield (kg/da) - determined by mowing, weighing grass in replicates for each harvest plot with subsequent drying of samples to constant weight at 105ºC and recalculated for 1da;

- Height (cm) - recorded by mowing at the time of harvest of the grass, along both diagonals of each plot, plants were measured at 4 points from the soil surface to the top of the tallest stems and averages calculated from the data;

**Climate characteristics in the experimental area**

The experimental territory belongs to the Pre-Balkan (mountain) climate region of the temperate-continental climate subregion (Sabev and Stanev, 1963). The average annual temperature is characterized by territorial differentiation (from north to south) with increasing altitude. The average of the annual temperatures is 10/11°C (Ninov, 1997). The distribution of precipitation is uneven with a maximum in summer (309 mm) and minimum (168 mm) in winter. Spring is relatively cool and well-supplied with rainfall.

During the experimental period, the average annual air temperatures were 0.4ºC to 2.0ºC higher compared to those of the multiannual period (10.6ºC) (Table 1).

The temperature values in March (3.2-7.8ºC) were suitable for germination and development of the studied forage species. The average temperature during the vegetation (March-October) was from 14.6ºC (2021) to 16.3ºC (2023) with an average for the experimental period of 15.3ºC and an average for a multiannual period of 14.8ºC.

The characteristic of weather during the experiment shows the variation of climate factors, which specifically affect the development, productivity, and quality of forage species.

Table 1. Average monthly air temperature (ºC) for the period 1990-2023

| Years | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | Average | Average for III-X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2020 | 0.4 | 4.4 | 7.1 | 9.4 | 14.7 | 17.8 | 20.4 | 21.1 | 17.8 | 12.7 | 5.2 | 3.8 | 11.2 | ***15.1*** |
| 2021 | 1.6 | 3.7 | 3.6 | 8.3 | 15.4 | 18.9 | 22.7 | 22.7 | 16.2 | 8.7 | 7.5 | 2.6 | 11.0 | ***14.6*** |
| 2022 | 0.8 | 3.8 | 3.2 | 10.3 | 15.9 | 19.8 | 22.1 | 21.8 | 16.3 | 12.0 | 8.3 | 4.4 | 11.6 | ***15.2*** |
| 2023 | 5.3 | 3.2 | 7.8 | 9.9 | 14.2 | 19.0 | 23.2 | 22.3 | 18.8 | 15.4 | 8.1 | 3.9 | 12.6 | ***16.3*** |
| 2020-2023 | ***2.0*** | ***3.8*** | ***5.4*** | ***9.5*** | ***15.1*** | ***18.9*** | ***22.1*** | ***22.0*** | ***17.3*** | ***12.2*** | ***7.3*** | ***3.7*** | ***11.6*** | ***15.3*** |
| 1990-2019 | ***-0.5*** | ***2.3*** | ***5.5*** | ***10.5*** | ***15.2*** | ***18.8*** | ***20.8*** | ***20.7*** | ***15.7*** | ***10.9*** | ***6.1*** | ***1.8*** | ***10.6*** | ***14.8*** |

The highest annual precipitation amount (712.9 mm) were reported in 2023 compared to the other experimental years, but also compared to the average annual norm for the period 1990-2019 (789.7 mm), the values are 76.8 mm lower (Table 2). The data indicate that the annual precipitation amount during the experimental period is lower by 33.3-167.6 mm compared to those for a multiannual period. Droughts in Bulgaria are observed in all seasons, which affects the physiological processes during the different phenophases of the individual development of forage species.

The lowest annual precipitation amount (545.3 mm) and vegetation precipitation amount (March-October - 379.9 mm) were registered in the third experimental year (2022) when the main components in the monoculture and mixed grasslands reached optimal development and increased their participation in the grassland.

Table 2. Monthly and annual precipitation amount (mm) for 1990-2023

| Years | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | Annual amount | Amount for III-X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2020 | 15.4 | 66.2 | 53.4 | 24.4 | 63.8 | 129 | 75.4 | 56.4 | 33.6 | 114.2 | 20.4 | 27.4 | ***679.6*** | ***550.2*** |
| 2021 | 82.8 | 25.6 | 47.7 | 57.0 | 82.8 | 64.8 | 12.4 | 56.2 | 11.8 | 72.8 | 23.6 | 68.6 | ***606.1*** | ***405.5*** |
| 2022 | 21.8 | 55.4 | 14.4 | 95.8 | 28.8 | 78.9 | 35.4 | 64.6 | 58.8 | 3.2 | 61.6 | 26.6 | ***545.3*** | ***379.9*** |
| 2023 | 12.4 | 27.8 | 25.8 | 82.6 | 174.5 | 132.4 | 27.6 | 50.2 | 28.4 | 1.8 | 81.2 | 68.2 | ***712.9*** | ***523.3*** |
| 2020-2023 | ***33.1*** | ***43.8*** | ***35.3*** | ***65.0*** | ***87.5*** | ***101.3*** | ***37.7*** | ***56.9*** | ***33.2*** | ***48.0*** | ***46.7*** | ***47.7*** | ***636.0*** | ***464.7*** |
| 1990-2019 | ***41.6*** | ***40.6*** | ***56.7*** | ***66.9*** | ***98.2*** | ***111.8*** | ***98.0*** | ***66.7*** | ***69.5*** | ***58.0*** | ***37.5*** | ***44.4*** | ***789.7*** | ***625.6*** |

The sum of the annual and vegetation precipitation is lower by 153.7 mm and 160.9 mm, respectively, compared to the multiannual period. Air temperature and precipitation are factors impacting the composition, density, and resistance of the studied plant species. In the experimental years, spring moisture offered optimal conditions for the formation of the first regrowth, both in monoculture and mixed grasslands.

*Analysis Toolpak for Microsoft Excel 2010* and *Statgraphics Plus v.2.1* software were used for statistical data processing.

## RESULTS AND DISCUSSION

**Yield of fresh and dry mass from individual stands of grass forage crops and their binary mixtures with *Trifolium pratense* L.**

Given the global warming climate and to minimize the negative environmental impact on forage productivity and quality, it is challenging to establish forage crop species with high adaptive potential in areas with variable and uneven rainfall distribution (Churkova, 2013; Huang *et al.*, 2017; Ferner *et al.*, 2018; Churkova and Churkova, 2021).

Over the four-year test period, monoculture crops of grass forages recorded demonstrably higher productivity compared to the corresponding mixtures (Figures 1 and 2).

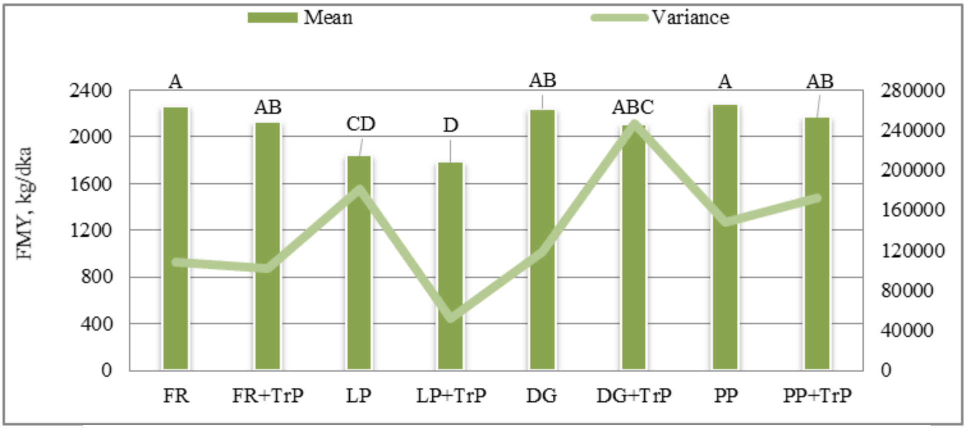


Figure 1. Fresh mass yield (kg/da) of grasslands with monoculture grass species and in mixtures with *Trifolium pratense* L. (average for the period 2020-2023)

The highest yields of fresh (2279.2 kg/da) and dry mass (929.3 kg/da) were recorded for the sole crop of *Phleum pratense* L., followed by the variants of *Dactylis glomerata* L. (2241.7 kg/da - fresh mass and 873.9 kg/da - dry mass). For the experimental period, the presence of the forage crop in the two grass stands was over 90%, which is an indication of good resistance and adaptability of the plants on the pseudopodzolic soils in the experimental area. The fresh mass yield in the monoculture crops of *Phleum pratense* L. and *Dactylis glomerata* L. was higher by 110.5 kg/da and 140.0 kg/da, respectively, compared to their two-component mixtures. There was a significant difference in dry mass yield between the two types of grasses. The values of the index in the mixtures of *Phleum pratense* L. with *Trifolium pratense* L. and *Dactylis glomerata* L. with *Trifolium pratense* L. were lower by 31.3% and 17.4%, respectively ($P<0.05$), compared to the monoculture grasslands of the respective grass species.

For the study period, the pure crops of *Lolium perenne* L. had a proven higher dry mass productivity compared to the mixture of this species with *Trifolium pratense* L. The excess in the values of the indicator was 20.7%. In the years of study, *Lolium perenne* L. dominated the mixed grass stand and was the main component forming the harvested forage yield (Bozhanska *et al.*, 2024).

Analysis of the data indicated that the grassland had the lowest yield (< 20%) of fresh (1789.6 kg/da) and dry mass (517.1 kg/da) compared to the average values of the parameters in the binary mixtures included in the study.

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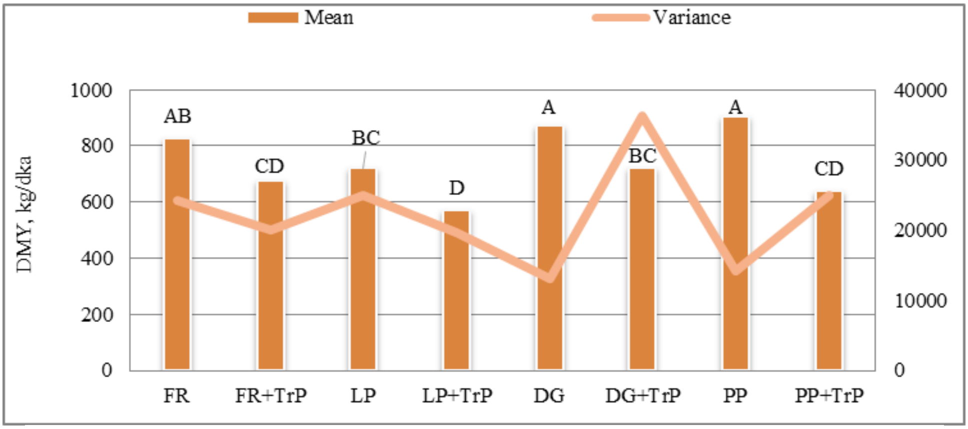


Figure 2. Dry mass yield (kg/da) of grasslands with monoculture grass species and in mixtures with *Trifolium pratense* L. (average for the period 2020-2023)

The monoculture grasslands with *Festuca rubra* L. had a higher fresh (2258.3 kg/da) and dry (825.0 kg/da) mass yield compared to the corresponding mixture. A proven excess in the amount of dry mass by 22.0% (P <0.05) was found.

On average over the period, monoculture crops of *Lolium perenne* L. were shown to have the lowest fresh mass (by 17.8% to 19.2%) and dry mass (by 17.6% to 22.5%) productivity compared to sole stands of *Festuca rubra* L., *Dactylis glomerata* L. and *Phleum pratense* L.

In the two-component mixtures, the excess in fresh mass yield was 17.7-21.2% (P <0.05) compared to the grasslands of *Lolium perenne* L. and *Trifolium pratense* L. It was found that in the conditions of the Middle Balkan Mountains, the mixture *Dactylis glomerata* L. + *Trifolium pratense* L. exceeded the dry mass yield in the grasslands of *Lolium perenne* L. + *Trifolium pratense* L. by 26.4% (P <0.05).

**Height of grass and legume component grown in sole and mixed stands**

The height of the components in the grass stand provides information related to the sole and combined use of perennial grass and legume forage grasslands, assesses their compatibility, stability and productivity under the prevailing unfavourable soil and climatic conditions in upland areas.

Height of grass species

The average plant height in single crops of *Festuca rubra* L. varies from 38.1 cm to 80.2 cm (Figures 3-6). The values are 2.7 to 14.3 cm higher than in the corresponding two-component mixture. Grass crop growth in both types of grasslands (monoculture and mixed) was highest in the third growing season (2022), when *Festuca rubra* L. also reached maximum productivity. In the fourth growing season, with a more significant difference in grass height, mixed grasslands with *Trifolium pratense* L.

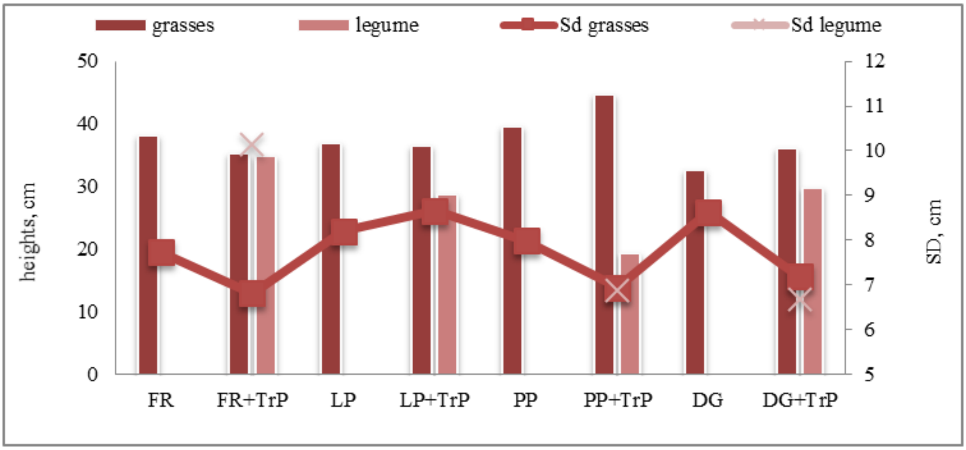


Figure 3. Heights (cm) of grass species and legume component in monoculture and mixed grasslands (first experimental year)

*Lolium perenne* L. is a valuable forage crop with good development in both upland and lowland conditions (Petkova *et al.*, 2021).

In the year of sowing, only the stems of *Lolium perenne* L. had almost identical values for the plant height trait in mixed and monoculture crops. The data indicate a minimal difference (36.6-36.9 cm) in the height of the grass in the two types of grasslands (single and mixed).

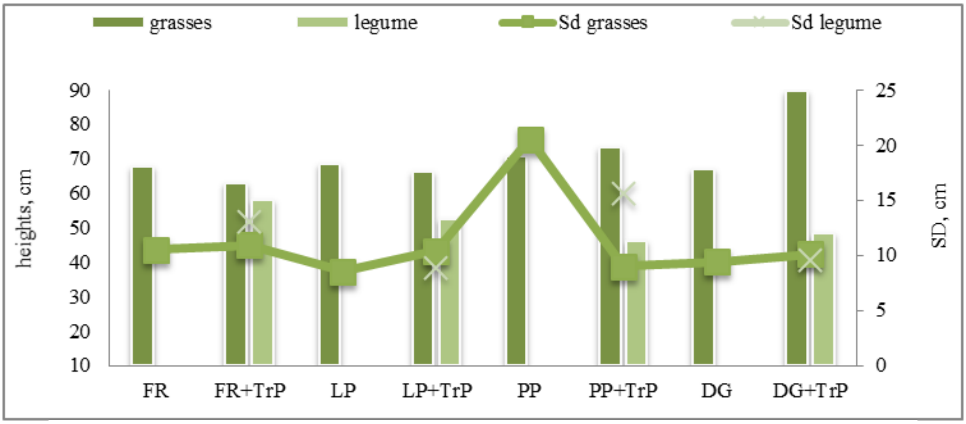


Figure 4. Heights (cm) of grass species and legume component in sole and mixed grasslands (second experimental year)

The association of *Lolium perenne* L. with *Trifolium pratense* L. as well as the increase in the age of the grasslands resulted in a more significant difference in stem height in the grass. In the monoculture grasslands, the mean value of the indicator ranged from 68.4 to 84.8 cm, and in the corresponding mixture from 66.1 to 81.5 cm. According to Katova and Vulchinkov, (2019), perennial ryegrass plants vary strongly in habit and height.

The height of *Phleum pratense* L. grasslands was found to be positively correlated with fresh biomass productivity (r=0.78**) (Janković *et al.*, 2018). According to the results obtained, plants of the species *Phleum pratense* L. recorded lower values in stem height in single grasslands, compared to mixed ones, in the period from the first to the third vegetation. In contrast, in 2023 (the fourth growing season, the year with the highest annual rainfall), *Phleum pratense* L. plants in the monoculture crop were 6.6 cm taller than those in the corresponding mixture (73.0 cm), suggesting higher fresh biomass productivity.

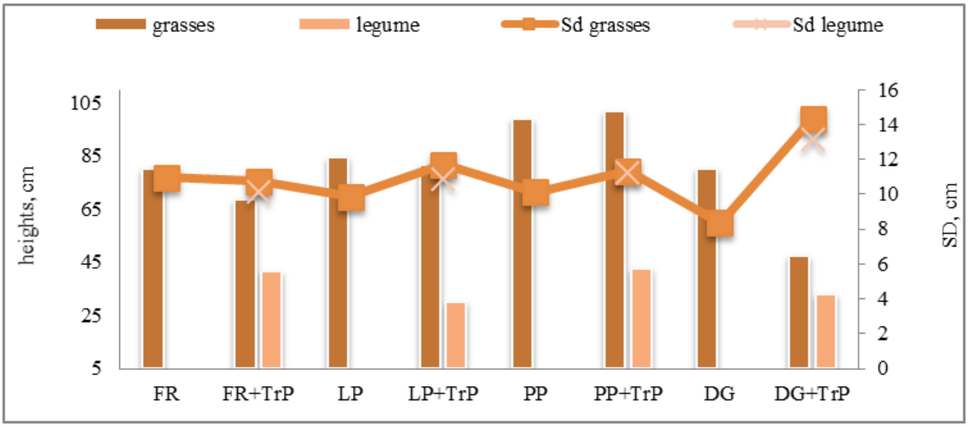


Figure 5. Heights (cm) of grass species and legume component in sole and mixed grasslands (third experimental year)

*Dactylis glomerata* L. is a grass forage crop with a strong competitive ability reflected by the values of the biometric indicator plant height (Bozhanska and Churkova, 2019). Data from the analysis indicate that plant height in monoculture cultivation ranged from 32.7 to 80.2 cm, and in mixed grasslands from 36.2 to 89.7 cm. In the first and second growing seasons, the plants of *Dactylis glomerata* L. recorded 3.5 to 22.7 cm lower values in stem height when grown alone compared with the two-component mixtures.

Dactylis glomerata L. is a perennial grass species, drought-resistant and with good productivity when grown in pure or mixed crops (Zhouri *et al.*, 2019). Interestingly in this case, in the year with the lowest rainfall amounts (2022), the difference in plant growth rate between the two grassland types is significant. The stems of *Dactylis glomerata* L. in the stand-alone grasslands were 32.9 cm taller than those in the corresponding two-component mixture (47.3 cm).

In the conditions of the Middle Balkan Mountains, grass species (the exception is *Dactylis glomerata* L. grown in a two-component mixture) in both types of grasslands are characterized by the highest rate of regrowth and emergence in the third growing season (2022 – is with the lowest amount of precipitation), which is a good indicator of their drought tolerance.

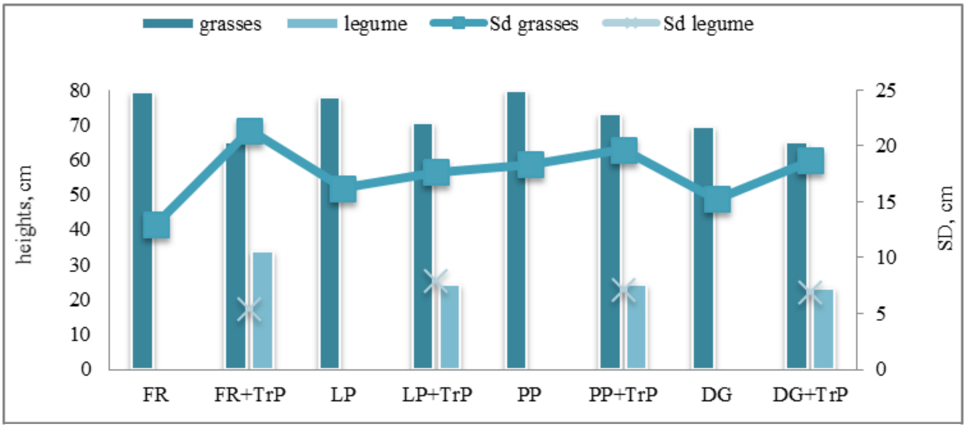


Figure 6. Heights (cm) of grass species and legume component in sole and mixed grasslands (fourth experimental year)

The average stalk height of forage grass species grown in sole grasslands and binary mixtures is:

- *Festuca rubra* L. – 66.3 cm and 58.0 cm;
- *Lolium perenne* L. – 66.9 cm and 63.7 cm;
- *Phleum pratense* L. – 72.2 cm and 73.2 cm;
- *Dactylis glomerata* L. – 62.3 cm and 59.6 cm.

**Height of legume component**

The good moisture supply during the first year of the experiment helped the growth rate of *Trifolium pratense* L., which is a mesophytic species.

The data show that the average height of *Trifolium pratense* L. in the binary mixtures ranged from 19.5 cm (*Phleum pratense* L. + *Trifolium pratense* L.) to 34.9 cm (*Festuca rubra* L. + *Trifolium pratense* L.). In the mixtures with *Lolium perenne* L. and *Dactylis glomerata* L. the values were 28.7 cm and 29.8 cm, respectively. In mixed cultivation of two or more species there are aspects of both intraspecific and interspecific competition (Razec and Razec, 2006). In the first growing season, the difference in the growth rate of the legume component depending on the grass species with which it is associated is significant. As an explanation for what was observed, the rapid rate of outgrowth and the greater competitive ability of grass can be pointed out, which suppresses the development of *Trifolium pratense* L., characterized by a slower rate of outgrowth in the year of sowing.

As a representative of the second cenotic group, *Trifolium pratense* L. demonstrates high competitive ability and the length of generative stems in the second vegetation reach the highest values in all variants. The trend characterising the minimum and maximum value of the legume component in the mixtures with *Phleum pratense* L. (46.3 cm) and *Festuca rubra* L. (57.9 cm) was also maintained. For the same period, the association of *Trifolium pratense* L. with *Lolium perenne* L. and *Dactylis glomerata* L. resulted in the formation of grassland where the mean height of the legume component was 52.4 cm and 48.3 cm, respectively.

The biological characteristics of the individual species as well as their interrelationships in mixed crops influence their longevity and purpose. In the conditions of the Middle Balkan Mountains, with increasing age of the stand, there is a delay in the development and growth process of *Trifolium pratense* L. In the third growing season, the length of generative stems in the legume component was above 30.0 cm in the mixtures with *Dactylis glomerata* L. (32.8 cm) and *Lolium perenne* L. (30.2 cm) and above 40.0 cm in the mixtures with *Phleum pratense* L. (42.9 cm) and *Festuca rubra* L. (41.8 cm).

In the fourth growing season, the legume crop recorded lower values for the plant height trait. The average height of *Trifolium pratense* in the two-component mixtures with *Festuca rubra* L. and *Phleum pratense* L. was 33.7 cm and 23.0 cm, respectively. Mixed grasslands of *Lolium perenne* L. (24.1 cm) and *Phleum pratense* L. (24.4 cm) had minimal difference in the values of the indicator.

In the conditions of the Middle Balkan Mountains, the mixture with the best uniformity in the height of the grass and legume component was the binary mixture of *Festuca rubra* L. + *Trifolium pratense* L. With the greatest difference in the mean values of the trait was the mixture of *Phleum pratense* L. + *Trifolium pratense* L.

**Significance of factorial influence on productivity and height of sole and mixed grass and legume forage grasslands**

The factors - environmental conditions, age and type of grassland had a significantly influenced ($P < 0.01$–$P < 0.001$) the values of the studied traits (Table 3).

Year and age ($\eta^2$ ranged from 14.7 to 67.6%) and type of herbage ($\eta^2$ ranged from 2.4 to 40.3%) significantly influenced forage crop productivity and height.

Table 3. Degree ($\eta^2$) and significance (P) of factorial influences on yield and height of monoculture forage grass grasslands and their mixtures with *Trifolium pratense* L.

| Sources of variation / Signs | Year and age of grassland | | Type of grasslands | | Interaction | |
|---|---|---|---|---|---|---|
| | $\eta^2$ (%) | P | $\eta^2$ (%) | P | $\eta^2$ (%) | P |
| FMY-monocul. grasslands | 14.7 | P <0.01 | 21.3 | P <0.001 | 29.9 | ns |
| FMY- mixed grasslands | 28.0 | P <0.001 | 19.3 | P <0.01 | 15.5 | ns |
| DMY- monocul. grasslands | 21.1 | P <0.01 | 21.8 | P <0.01 | 19.0 | ns |
| DMY- mixed grasslands | 61.2 | P <0.01 | 11.8 | P <0.01 | 3.9 | ns |
| Hight – monocul. grasslands | 67.6 | P <0.001 | 2.4 | P <0.001 | 1.8 | P <0.001 |
| Hight - mixed grasslands | 21.7 | P <0.001 | 40.3 | P <0.001 | 14.6 | P <0.001 |

The interaction between the factors under study accounted for a significant proportion of the factorial variance in the traits plant height in the sole ($\eta^2$=1.8%) and mixed grasslands ($\eta^2$=14.6%). According to the results obtained, the factorial interaction had insignificant influence on the traits of fresh and dry mass yield for both types of grasslands.

## CONCLUSIONS

In the conditions of the Middle Balkan Mountains, the independent stands of *Phleum pratense* L. have been shown to have the highest yield of fresh (2279.2 kg/da) and dry matter (929.3 kg/da). The excess in the values of the indicators was up to 23.8% and 29.0%, respectively, compared to the other monoculture grass crops studied. In the two-component mixtures, the grassland of *Phleum pratense* L. with *Trifolium pratense* L. (2168.7 kg/da) and *Dactylis glomerata* L. with *Trifolium pratense* L. (722.2 kg/da) had the highest fresh and dry mass yields.

On average over the period, the stem height of the forage grass species in single grasses ranged from 32.7 cm to 99.2 cm, and in their two-component mixtures from 35.4 cm to 101.9 cm. The two-component mixture of *Festuca rubra* L. with *Trifolium pratense* L. had the most optimal evenness in the height of the wheat and bean components, as an indication of good compatibility under upland conditions.

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# IN SITU CONSERVATION OF PLANT BIODIVERSITY IN HOME GARDENS (CASE STUDY OF TWO VILAGES IN CENTRAL ALBANIA)

## SUMMARY

Home gardens play an important role in the overall agricultural system. In addition to providing helpful information that can support and encourage local communities to develop their home gardens sustainably as a form of in situ management and conservation of plant biodiversity, the study seeks to understand the significant role and benefits of home gardens for the well-being of rural communities. In order to preserve these natural resources, a variety of data was obtained via field observations on the issues related to the use of plants and the variety of agricultural species found in the farms and home gardens of two villages in the central Albania. A total of 47 plant species, belonging to 29 families, were identified from field observations in the home gardens of the 82 farmers interviewed. Important plant species, such as *Sideritis raiseri* Boiss and Heldr, categorized as critically endangered by the IUCN Red List; *Origanum vulgareae* L. and *Juglans regia* L., categorized as endangered; *Salvia officinalis* L. and *Prunus persica* L. as vulnerable; and *Crataegus heldreichii* as low-risk, were also recorded in the home gardens under study. The outcome implies that there is a considerable degree of variability or variation in the plant species that the farmers have grown in their home gardens. These findings indicate that home gardens are suitable places for the conservation of these plants.

**Keywords**: biodiversity, conservation, farmers, home gardens, management

## INTRODUCTION

A vital component of farmers' livelihood strategy is genetic variety, particularly in regions with high ecological, climatic, and economic risks and pressures. Agro-biodiversity, as defined by various studies (Negri and Polegri

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2009), is a subset of natural biodiversity that comprises plant genetic resources (crops, cultivars, weeds, and wild relatives) used for food and agriculture. Different studies suggest that the maintenance of genetic variation within crops (Tilman, 2000; Xhulaj and Gixhari 2020) provides a wide range of essential goods and services that support ecosystem functioning and has become an important element of sustainable agriculture (Paoletti, 2001; Le Coeur *et al.* 2002; Marshall and Moonen 2002). Additionally, farmers and breeders can select and continuously modify crops to meet changing environmental conditions (Xhulaj and Koto 2022) or to meet the demands of an expanding human population (Bode *et al.* 2013; IPGRI 1993) thanks to the raw materials provided by agrobiodiversity. Home gardening is a type of traditional conservation where farmers in the area produce some valuable plant species close to their homes (Galluzi *et al.* 2010). They have been the subject of numerous studies that have examined their capacity to support biodiversity or reduce poverty (Reyes-Garcia *et al.* 2010; Fraser *et al.* 2011; Salako *et al.* 2014). It's unclear, though, if the locals still possess the expertise needed to maintain this system. This study aims to contribute modestly to the addition of useful information that can support and encourage local communities to develop their home gardens sustainably. It recognizes the important role and benefits of home gardens for the well-being of the rural community, as well as the paucity of information currently available on home gardens in Albania.

## MATERIAL AND METHODS

**Study area:** Roshnik and Qafë-Dardhë, two villages in the Berat District, were the study's locations. The village of Roshnik is situated in a region with latitude 40°43'53.29'N and longitude 20°2'32.56'E, roughly 18 km from the city of Berat. The village of Qafë Dardhë is situated in a region with latitudes of 40°44'31.37'N and longitudes of 20°7'12.10'E.

**Data collection:** The period for collecting information in 2023 was April through August. Eighty-two farmers-45 from Qafë-Dardhë and 37 from Roshnik village, were questioned in total from both villages. The study's participants were chosen at random (Table 1). Farmers shared information using a semi-structured questionnaire meant to gather information on a range of topics. They accompanied the researcher to the field for plant identifications after the interviews were completed. Participants initially used their colloquial names to identify the plants.

**Data Analysis:** The consistency and depth of the data were meticulously examined. In the analysis, descriptive statistics such as percentage and frequency were employed using XLSTAT software. The Jaccard Index (Jaccard, 1912), commonly referred to as the Jaccard similarity coefficient, was determined using the quantitative data that were gathered. It is defined as the size of the joint divided by the size of the union of the sample sets and is used to quantify the similarity and diversity between small groups of samples. As stated, (the number in both sets) / (the number in either set) *100 is the Jaccard Index.

Table 1. Sociological characteristics of the study sample

| Category | % of representation for the Village | |
|---|---|---|
| | **Qafë-Dardhë** | **Roshnik** |
| **Age** | | |
| 15-30 | 6.66 | 8.01 |
| 31-45 | 13.33 | 10.81 |
| 45-60 | 35.55 | 56.75 |
| 61-75 | 35.55 | 24.32 |
| ≥ 75 | 8.88 | - |
| **Gender** | | |
| Men | 55.5 | 67.56 |
| Women | 44.5 | 32.44 |
| **Main Activity** | | |
| Agriculture | 100 | 75.67 |
| Private company | - | 5.41 |
| Public administration | - | 18.91 |
| **Level of education** | | |
| Primary school | 91.1 | 40.54 |
| High school | 2.22 | 27.02 |
| University degree | 6.66 | 32.43 |

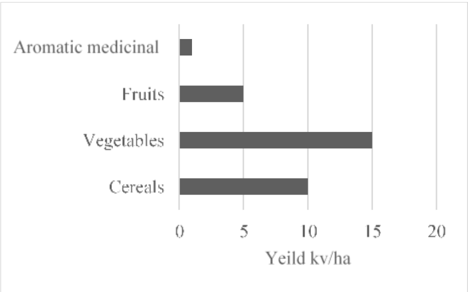
## RESULTS AND DISCUSSION

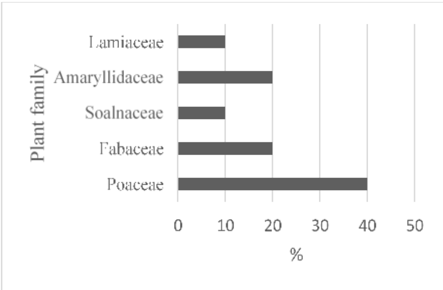
It is crucial to comprehend the social traits of farmers to comprehend their perspectives and adaptation strategies. According to different studies (Ball, 2020), gender reveals the roles that men and women play in rural communities and aids in determining the proportion of each group's involvement in agriculture. Women in agriculture, play a significant role in managing animals, processing and preparing food, trading agricultural and livestock crops, and maintaining families and homes. According to our findings, 44.5% of the farmers questioned in the village of Qafë-Dardhë were women (Table 1), a level of 32.44% participation had a representative percentage higher than that of Roshnik village. Farmers' years of experience in agriculture have a significant impact on how they perceive and make decisions in their day-to-day agricultural activities, including choosing the right cultivar, treating the soil, and meeting other requirements for producing as safely as possible (Zhou D and Li L 2022). In the village of Qafë-Dardhë, the majority of our respondents (71%) had over 41 years of experience in the agricultural field (Table 1.). This was followed by periods of 10 to 30 years, where 11.1% of respondents had experience, and 6.66% of our farmers had 11 to 20 years of experience.

### Diversity of plant species cultivated in the farms of the two villages under study

The possibility for farmers to improve their standard of living and their income stream is correlated with the availability of agricultural land. The chance of climate change adaptation is also influenced by the size of the farm. A large farm can help spread the risk of erratic weather fluctuations and offer opportunities for crop diversification (Belay *et al.* 2017).

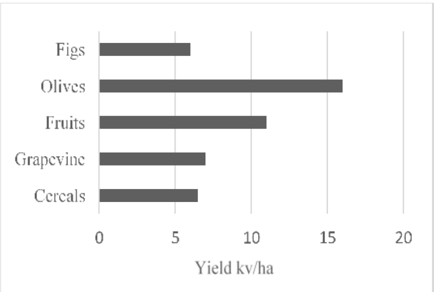
Based on field observations and data collected from study participants, it can be concluded that all farmers in Qafë-Dardhë village (100%) possess a small plot of land ranging from 0 to 1 hectare. In the meanwhile, compared to the first village, where land ownership ranges from 1 to 3 ha, farmers in Roshnik village (100%) own a bigger portion of the land. Four categories of crops (Figure 1) were produced by the farmers in the village of Qafë-Dardhë as their primary agricultural output: cereals, fruits, and vegetables. More recently, the cultivation of fragrant therapeutic plants has been introduced.

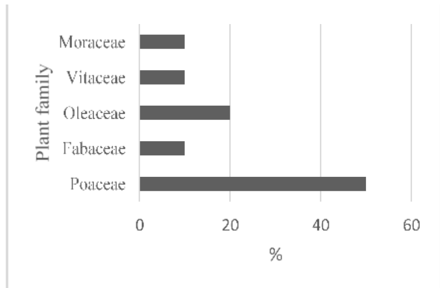



(a) (b)

Figure 1. (a) Data on the main products' average yields planted on agricultural lands of Qafë-Dardhë farms. (b) Data on the families of the main crops




(a) (b)

Figure 2. (a) Data on the primary crop average yields cultivated on Roshnik farms' agricultural area. (b) Data about the families of the principal crops

According to the findings of the field observations conducted with the farmers in Roshnik village, Table 2 shows that the three primary crop types planted on their property were grains, fruits, and vegetables.

At the same time, other plots containing grapevines, olives, and figs are developing. The highest yield was obtained from olives cultivated for oil production, followed by various fruits and then cereals (Figure 2).

Table 2. Data on the primary crops planted in the agricultural lands of the farms under study

| **Scientific name** | **Vernacular name** | **Family** | **Habitat/ life form** | **Main Use** |
|---|---|---|---|---|
| **Data on the primary crops planted in Qafë-Dardhë farms** | | | | |
| *Triticum sp.* L | Wheat | Poaceae | Herb | Commercial |
| *Avena sativa* L. | Oat | Poaceae | Herb | Commercial |
| *Hordeum vulgareae L.* | Barley | Poaceae | Herb | Commercial |
| *Zea mays* L. | Corn | Poaceae | Herb | Commercial |
| *Phaseolus vulgaris* L. | Beans | Fabaceae | Herb | Commercial |
| *Solanum tuberosum* L. | Potatoes | Solanaceae | Perennial plant | Commercial |
| *Allium cepa* L. | Onion | Amaryllidaceae | Perennial plant | Commercial |
| *Medicago sativa* L. | Alfalfa | Fabaceae | Perennial plant | Commercial |
| *Allium sativum* L. | Garlic | Amaryllidaceae | Perennial plant | Commercial |
| *Sideritis raiseri* | Mountain tea | Lamiaceae | Perennial plant | Commercial |
| **Data on the primary crops planted in Roshnik farms** | | | | |
| *Triticum sp.* L | Wheat | Poaceae | Herb | Commercial |
| *Olea europea* L. | Olive | Oleaceae | Tree | Commercial |
| *Avena sativa* L. | Oat | Poaceae | Herb | Commercial |
| *Hordeum vulgareae* L. | Barley | Poaceae | Herb | Commercial |
| *Zea mays* L. | Corn | Poaceae | Herb | Commercial |
| *Phaseolus vulgaris* L. | Beans | Fabaceae | Herb | Commercial |
| *Vitis vinifera* L. | Grapevine | Vitaceae | Shrub | Commercial |
| *Ficus carica L.* | Fig | Moraceae | Tree | Commercial |

Source of crop seeds or seedlings for field planting: farmers for most of the previously mentioned plant groups admitted that, in Roshnik village, the largest percentage (70%) obtained their seed from agricultural pharmacies as certified seeds; in contrast, at least two plant groups, vegetables and cereals the farmers got their seeds from previous planting seasons through annual seed exchanges. In contrast, 60% of the planting material utilized in the lands of farms in the village of Qafë-Dardhë was native, and it was shared among farmers season after planting season.

**Diversity of plant species in the home gardens of Qafë-Dardhë village**

Our results are based on 45 residential gardens of various sizes that encircle homes. The majority of cases (73.3%) have gardens that range in size from 1000 to 1500 m$^2$, followed by 17% with gardens that range in size from 700 to 1000 m$^2$, and 6.66% of family gardens have sizes less than 700 m$^2$ (400-700m$^2$). A vast array of plants, ranging in size from tiny herbs to trees, can be found in home gardens. From the field visit to 45 home gardens, 41 species belonging to 18 families were recorded for this study (Table 3, Figure 3).

The number of species in each family as well as the representative families were determined. The family *Lamiaceae* has 9.75% of its species, whereas the *Rosaceae* family has the most at 26.82 %. The families of *Brassicaceae* and *Amaranthaceae* comprise 4.87% of species, while the families of *Solanaceae*, *Cucurbitaceae*, *Apiaceae*, and *Fabaceae* account for 7.31% of species each. The remaining families are represented by 2.43% of species each (Table 3, Figure 3).

A number of useful plant species that are planted for personal use are noted in home gardens and can be found in the forests surrounding farms. Some of these species, like *Crataegus heldreichii*, *Salvia officinalis* L., and *Sideritis raiseri* Boiss and Heldr, are used medicinally by farmers and are listed on the country's red list following IUCN criteria (FAO, 2016).

Table 3. Floristic information of 45 home gardens in Qafë Dardhë village

| Scientific name | Vernacular name | Family | Life form | Main Use |
|---|---|---|---|---|
| *Chenopodium album* L | Wild spinach | Amaranthaceae | Herb | Food |
| *Spinacia oleracea* L. | Spinach | Amaranthaceae | Herb | Food |
| *Daucus carota* L. | Carrots | Apiaceae | Shrub | Food |
| *Petroselinum crispum* L. | Parsley | Apiaceae | Herb | Food supplement |
| *Anethum graveolens* L. | Dill | Apiaceae | Herb | Food supplement |
| *Lactuca sativa* L. | Salad | Asteraceae | Shrub | Food |
| *Corylus avellana* L. | Hazelnut | Betulaceae | Shrub | Food |
| *Brassica sp.* | Cabbages | Brassicaceae | Shrub | Food |
| *Brassica oleracea* L. | Wild cabb. | Brassicaceae | Shrub | Food |
| *Cucumis sativus* L. | Cucumber | Cucurbitaceae | Shrub | Food |
| *Citrullus lanatus* Thunb. | Watermelon | Cucurbitaceae | Shrub | Food |
| *Cucumis melo* L. | Melon | Cucurbitaceae | Shrub | Food |
| *Cornus mas* L. | Cornel | Cornaceae | Tree | Tea, Liquor |
| *Diospyros lotus* L. | Persimmon | Ebenaceae | Tree | Food |
| *Phaseolus vulgaris* L. | Bean | Fabaceae | Shrub | Food |
| *Pisum sativa* L. | Peas | Fabaceae | Shrub | Food |
| *Vicia faba* L. | Fava bean | Fabaceae | Shrub | Food |
| *Sideritis raiseri* * | Mountain tea | Lamiaceae | Herb | Medicines |
| *Origanum vulgare* L** | Oregano | Lamiaceae | Herb | Spice, tea |
| *Salvia officinalis* L*** | Sage | Lamiaceae | Shrub | Medicines |
| *Mentha piperita* L. | Mint | Lamiaceae | Herb | Spice |
| *Abelmoschus esculentus* | Okra | Malvaceae | Shrub | Food |
| *Ficus carica* L. | Fig | Moraceae | Tree | Food |
| *Primula veris* L. | Coweslips | Primulaceae | Herb | Tea, Liquor |
| *Crataegus heldreichii* | Hawthorn | Rosaceae | Tree | Medicines |
| *Malus sieversii* .Roem. | Wild apple | Rosaceae | Tree | Medicines |
| *Prunus spinosa* L. | Wild plum | Rosaceae | Tree | Tea, Liquor |
| *Malus domestica* Borkh. | Apple | Rosaceae | Tree | Food |
| *Pyrus species* L. | Pear | Rosaceae | Tree | Food |
| *Prunus avium* L.*** | Cherry | Rosaceae | Tree | Food |
| *Prunus persica* L. | Peach | Rosaceae | Tree | Food |
| *Solanum lycopersicum* L. | Tomato | Solanaceae | Shrub | Food |
| *Prunus amygdalus* L. | Almond | Rosaceae | Tree | Food |
| *Cydonia oblonga Mill.* | Quince | Rosaceae | Tree | Food |
| *Prunus domestica* L. | Plum | Rosaceae | Tree | Food |
| *Fragaria vesca* L. | Strawberries | Rosaceae | Shrub | Food |
| *Capsicum annuum* L. | Pepper | Solanaceae | Shrub | Food |
| *Solanum melongena* L. | Eggplant | Solanaceae | Shrub | Food |
| *Urtica dioica* L. | Nettles | Urticaceae | Herb | Food supplement |
| *Juglans regia* L.** | Walnut | Juglandaceae | Tree | Food |
| *Vitis vinifera* L. | Grape vine | Vitaceae | Shrub | Food |

**Critically endangered, ** Endangered, ***Vulnerable. (FAO, 2016)*

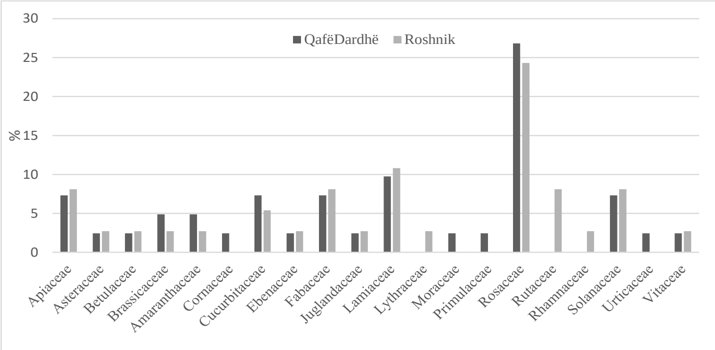
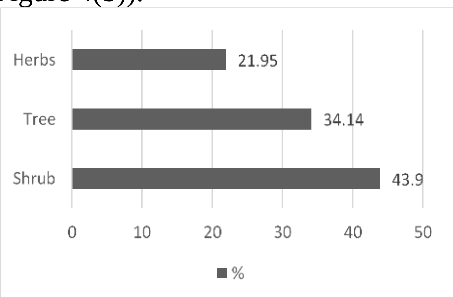


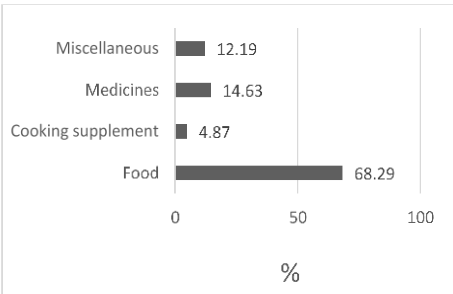

Figure 3. Species families represented in the home gardens of 82 farms

Proper dynamics have been established in home gardens by the integration of several plant species with varying life forms, such as trees, shrubs, and herbs. According to reports in the literature, most home gardens consist of a variety of plants with slightly variable compositions and levels of dominance. In accordance with other studies (Sujarwo and Caneva 2015), climbers, shrubs, and herbs were the most common plants in residential gardens, after trees. According to Barbhuiya *et al*. 2016, trees dominated residential gardens, with shrubs and plants coming in second and third. As previously reported (Mekonen *et al.* 2015) on botanical study of home gardens, herbs dominated, followed by trees, shrubs, and climbers.

**Diversity of plants life forms and their use in Qafë-Dardhë village**

The environment benefits from the interactions between plants and other living beings. According to reports, trees can increase soil nutrient availability through litter fall, absorb rainfall, move water from the soil to the atmosphere through transpiration, and reduce irradiance through shading (Holmgren *et al.* 2015). In our survey, shrubs accounted for 43.9% of the plant species grown in home gardens, followed by trees (34.14%) and herbaceous plants (21.95%) (Figure 4(a)). Farmers used them for personal consumption as food in 68% of the cases, while those with aromatic medicinal origins accounted for 14.6% of the cases, in the form of extract to treat flu, kidney-related diseases, etc. In 4.8% of cases, farmers employed them as spices, to be added as such to different cuisines. (Figure 4(b)).




(a) (b)

Figure 4. (a) Diversity of plants life forms; (b) Use of home garden plants

Diversity of plant species in the home gardens of Roshnik village. Our results refer to 37 home gardens which are presented with similar sizes ranging from 500 to1000 $m^2$. Home gardens are represented by a wide variety of plants, varying in their life forms, from small herbs to trees. 37 species, belonging to 17 families, were identified during the field visit to 37 home gardens for this study (Table 4).

Table 4. Floristic data on the main species of home gardens in the 37 farms of the village of Roshnik

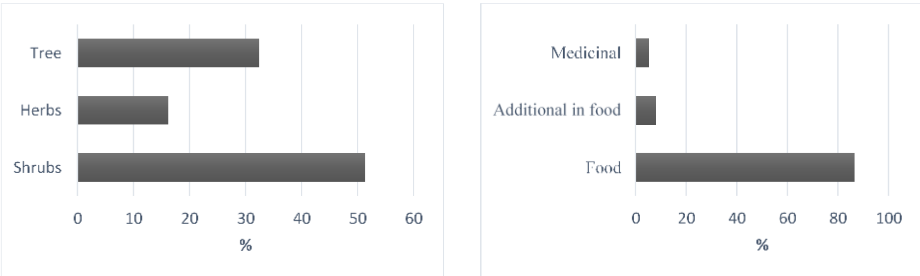
| **Scientific name** | **Vernicular name** | **Family** | **Life form** | **Main Use** |
|---|---|---|---|---|
| *Spinacia oleracea* L. | Spinach | Amaranthaceae | Herb | Food |
| *Petroselinum crispum* (Mill.) | Parsley | Apiaceae | Herb | Food supplement |
| *Anethum graveolens* L. | Dill | Apiaceae | Herb | Food supplement |
| *Daucus carota* L. | Carrots | Apiaceae | Shrub | Food |
| *Lactuca sativa* L. | Salad | Asteraceae | Shrub | Food |
| *Brassica sp.* | Cabbages | Brassicaceae | Shrub | Food |
| *Citrullus lanatus* (Thunb.) | Watermelon | Cucurbitaceae | Shrub | Food |
| *Cucumis sativus* L. | Cucumber | Cucurbitaceae | Shrub | Food |
| *Diospyros lotus* L. | Persimmon | Ebenaceae | Tree | Food |
| *Phaseolus vulgaris* L. | Bean | Fabaceae | Shrub | Food |
| *Pisum sativa* L. | Peas | Fabaceae | Shrub | Food |
| *Vicia faba* L. | Fava bean | Fabaceae | Shrub | Food |
| *Juglans regia* L.** | Wallut | Juglandaceae | Tree | Food |
| *Sideritis raiseri* Boiss & Heldr* | Mountain tea | Lamiaceae | Herb | Medicines |
| *Origanum vulgare* L.** | Oregano | Lamiaceae | Herb | Spice, tea |
| *Salvia officinalis* L.*** | Sage | Lamiaceae | Shrub | Medicines |
| *Mentha piperita* L. | Mint | Lamiaceae | Herb | Food supplement |
| *Punica granatum* L. | Pomegranate | Lythraceae | Shrub | Food |
| *Abelmoschus esculentus* L. | Okra | Malvaceae | Shrub | Food |
| *Ficus carica* L. | Fig | Moraceae | Tree | Food |
| *Ziziphus jujuba* Mill. | Ziziphus | Rhamnaceae | Tree | Food |
| *Prunus amygdalus* L. | Almond | Rosaceae | Tree | Food |
| *Cydonia oblonga* Mill. | Quince | Rosaceae | Tree | Food |
| *Prunus domestica* L. | Plum | Rosaceae | Tree | Food |
| *Fragaria vesca* L. | Strawberries | Rosaceae | Shrub | Food |
| *Malus domestica* Borkh. | Apple | Rosaceae | Tree | Food |
| *Pyrus species* L. | Pear | Rosaceae | Tree | Food |
| *Prunus avium* L.*** | Cherry | Rosaceae | Tree | Food |
| *Prunus persica* L. | Peach | Rosaceae | Tree | Food |
| *Mespilus germanica* L. | Medlar | Rosaceae | Shrub | Food |
| *Citrus × sinensis* (L.) Osbeck | Orange | Rutaceae | Tree | Food |
| *Citrus × limon* (L.) Osbeck | Lemon | Rutaceae | Tree | Food |
| *Citrus reticulate* | Mandarine | Rutaceae | Shrub | Food |
| *Capsicum annuum* L. | Pepper | Solanaceae | Shrub | Food |
| *Solanum lycopersicum* L. | Tomato | Solanaceae | Shrub | Food |
| *Solanum melongena* L. | Eggplant | Solanaceae | Shrub | Food |
| *Vitis vinifera* L. | Grape vine | Vitaceae | Shrub | Food |

**Critically endangered, ** Endangered, ***Vulnerable. (FAO, 2016)*

The representative families and the number of species in each family were identified, with the Rosaceae family having the highest percentage of species (24.9%), followed by the *Lamiaceae* family with 10.81%. Eight percent of species are represented by the families *Solanaceae*, *Apiaceae*, *Fabaceae*, and *Rutaceae*, respectively; 5.4% of species are represented by the *Cucurbitaceae* family, and 2.7% of species each by the remaining families (Table 4).

**Diversity of plants life forms and their use in Roshnik village.**

Three different life forms were represented among the plant species grown in home gardens: shrubs made up 51.35% of the cases, trees made up 32.43%, and herbaceous plants made up 16.22% (Figure 5(a)). In 86.48% of cases, the plant species were utilized by farmers for personal consumption as food; in 5.4% of cases, mostly those with medicinal aromatic origins, the plant species were used medicinally, typically in the form of extracts to cure illnesses like the flu. In 8.1% of the cases, farmers utilized them as spices to be added as such to different foods. (Figure 5(b)). Farmers surveyed for the study in both communities had similar views about the sections of the plant they used for their purposes, demonstrating that uses vary based on the type of plant.




(a) (b)

Figure 5: (a) Diversity of plant life forms (b) Use of home garden plants

The degree of diversity and similarity between the garden species groups of the two villages under study was measured using the Jacard coefficient, often known as the Jaccard Similarity Index (IJ). The two populations are more similar, the larger the percentage. For the plant spices that were displayed in the home gardens of the two villages that were the subject of the study, our computed value yielded an IJ of 39.74%. The outcome implies that there is a considerable degree of variability or variation in the plant species that the farmers have grown in their home gardens in the two communities. These findings indicate that home gardens are suitable places for the conservation of these plants.

## CONCLUSIONS

The results of our study, based on interviews and field observations, support the assertion that family gardens are important sources for maintaining agricultural plant biodiversity. In the 82 family gardens of the two villages selected for study, a total of 47 plant species belonging to 29 families were identified.

Meanwhile, in the agricultural lands, farmers mainly cultivated for commercial use about 14 plant crops, which belonged to 8 plant families. Important plant species, such as Sideritis raiseri Boiss & Heldr, categorized as critically endangered by the IUCN Red List; *Origanum vulgareae* L. and *Juglans regia* L., categorized as endangered; *Salvia officinalis* L. and *Prunus persica* L. as vulnerable; and *Crataegus heldreichii* as low-risk, were also recorded in the home gardens under study. Given the significant benefits and contribution of home gardens to human well-being and the dearth of research on home gardens in Albania, this study may provide useful information that might support and encourage locals to grow their home gardens in a sustainable manner.

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# SALINITY EFFECTS ON SEED GERMINATION OF TWO POTENTIAL HORTICULTURAL SPECIES (PHYSALIS PERUVIANA AND ARTEMISIA HERBA-ALBA)

## SUMMARY

Salinity stress presents a challenging encounter to seed germination, deeply impacting plant establishment, particularly in regions characterized by arid and semiarid conditions. This study investigates the effect of varying NaCl concentrations on the seed germination of two multipurpose plant species, *Artemisia herba-alba* and *Physalis peruviana*. Through controlled experimental protocols, we evaluated critical germination attributes, including final germination percentage (FGP), mean germination time (MGT), time to 50% germination ($T_{50}$) and germination tolerance index (GTI). After 22 days of saline treatment, our results revealed distinct responses for each species to salinity stress. *A. herba-alba* demonstrated a moderate sensitivity, with FGP declining from 67.3% at 0 mM NaCl to 10.7% at 200 mM NaCl, whereas *P. peruviana* exhibited remarkable tolerance, maintaining a consistently high FGP of 100% across all NaCl concentrations except for the concentration of 200 mM (36.7%). Statistical analysis employing two-way ANOVA underscore the significant main effects of salinity, species, and their interactions on seed germination parameters. This study highlights the imperative of understanding species-specific adaptive strategies to mitigate salinity-induced inhibitions on seed germination. These insights advance our comprehension of seed responses to environmental stress and hold implications for the conservation, cultivation, and management of *A. herba-alba* and *P. peruviana* in saline-affected ecosystems.

**Keywords**: *Artemisia herba-alba*, NaCl, germination attributes, salinity, *Physalis peruviana*

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## INTRODUCTION

An elevated concentration of soluble salts in soils has detrimental effects on agricultural lands, crops, and subsequently, the livelihoods of people worldwide. Over 100 nations are dealing with challenges related to soil salinity and the concurrent salinization of groundwater resources. Irrigation of agricultural crops with saline water indeed increases the concentration of soluble salts in soil, thereby reducing the productivity of crop plants (Srivastava *et al.*, 2019). Seed germination is affected by salinity stress, particularly in regions characterized by arid and semiarid conditions (Kigel, 2017; Kheloufi *et al.*, 2018; Christiansen *et al.*, 2022). Saline soils in arid rangelands of Algeria are primarily constituted by the accumulation of diverse chloride and sulfate salts, with NaCl predominating at over 50% (Halitim, 1988; Mansouri and Kheloufi, 2024). When salinity stress and drought interact, seeds are subjected to osmotic stress, reducing water uptake and metabolic processes needed to germinate. Salinity stress is intensified by drought by reducing soil moisture, which increases salt accumulation in root zones (Johal and Goyal, 2023). Consequently, the dual stress of drought and salinity hampers seed germination and negatively impacts crop growth, development, and productivity. Moreover, the accumulation of salts in the soil further reduces its fertility, leading to long-term degradation of agricultural land in arid and semiarid regions (Muhammad *et al.*, 2024).

*Artemisia herba-alba* Asso. (*Asteraceae*) (also called white wormwood) and *Physalis peruviana* L. (*Solanaceae*) (also called golden berry) possess a range of ecological, medicinal, and economic attributes. *A. herba-alba* is known for its anti-inflammatory, antimicrobial, and antioxidant properties, making it valuable in traditional medicine practices (Nedjimi and Beladel, 2015). P. peruviana is also valued for its medicinal properties, with various parts of the plant used to treat diseases such as inflammation, asthma, and gastrointestinal disorders (Ezzat and Salama, 2024). *P. peruviana* produces edible fruits enclosed in a papery husk, which are commonly consumed fresh or used in culinary applications such as jams, desserts, and salads (Cortés *et al*., 2012). The fruit is rich in vitamins, minerals, and antioxidants, contributing to its nutritional value and culinary versatility. It is cultivated commercially for its fruits, which are traded internationally and have economic value in fresh and processed forms (Obregón La Rosa, 2024). A. herba-alba possesses aromatic properties, with the plant emitting a distinctive fragrance due to its essential oil content. This aromatic quality has led to its use in perfumery, aromatherapy, and the production of essential oils (Fadel *et al.*, 2023). Both species are perennial and play significant roles in their respective ecosystems. *A. herba-alba* is known to have allelopathic effects, influencing the composition and dynamics of plant communities in its habitat (Arroyo *et al*., 2016). *P. peruviana*, on the other hand, serves as a food source for various wildlife species and contributes to ecosystem biodiversity.

Unfortunately, both species are subjected to diverse environmental stresses, including salinity, which significantly affect their growth, development, and yield potential (Nedjimi and Zemmiri, 2019; Aydin, 2024). In previous studies, *A. herba-alba* and *P. peruviana* have demonstrated significant horticultural potential due to their tolerance to salinity and drought, making them suitable for cultivation in arid regions. These characteristics position both species as valuable options for sustainable agriculture in areas facing water scarcity and soil salinity challenges (Nedjimi and Zemmiri, 2019; Muñoz *et al*., 2021). On the other hand, there is a scarcity of information regarding the ecophysiological factors influencing germination in these two species. Therefore, understanding the responses of key plant species, such as *A. herba-alba* and *P. peruviana*, to salinity stress is crucial for creating strategies to mitigate the adverse effects of salinity on crop production in these challenging environments. Indeed, previous studies have highlighted the detrimental impact of salinity on seed germination, attributing it to alterations in water uptake, osmotic potential, and ion imbalance within seeds (Nikolić *et al.*, 2023; Khan *et al.*, 2023). The sensitivity of seeds to salinity varies across species, with some revealing tolerance mechanisms such as osmotic adjustment, ion exclusion, and antioxidant defense systems (Johnson and Puthur, 2021). However, the comprehensive mechanisms underlying salinity tolerance during seed germination remain incompletely understood, requiring further investigation.

In this study, we aim to elucidate the salinity effects on seed germination of *P. peruviana* and *A. herba-alba*, focusing on key germination responses underlying their differential tolerance to salinity stress under varying salinity levels of sodium chloride. The findings from this study are expected to enhance our understanding of the adaptive mechanisms employed by these two species to cope with salinity stress during seed germination. Furthermore, the insights gained could have implications for the conservation, cultivation, and management of these species in salt-affected environments, contributing to sustainable agricultural practices and ecosystem resilience in the face of global environmental changes.

## MATERIAL AND METHODS

### Seed harvest and origin

Table 1 presents the provenances of the seeds used in this study for *Physalis peruviana* and *Artemisia herba-alba*. The table also presents seed biometric parameters for each species, including the 1000-seed weight, as well as seed length and width. The measurements were taken based on a sample of 100 seeds per species. Both seed species were collected on November 2023 from several individuals growing in apple orchard (Figure 1). For *P. peruviana*, the ripe fruits were selected, opened, and the seeds were extracted manually before being left to dry naturally for two weeks. Seeds of both species were then stored in paper bags at room temperature until their use on February 2024.

Table 1. Seed characteristics and origins of *Physalis peruviana* and *Artemisia herba-alba*.

| **Parameters** | ***Physalis peruviana*** | ***Artemisia herba-alba*** |
|---|---|---|
| **1000-seed weight (g)** | 0.11 | 0.21 |
| **Length (cm)** | 0.20 ± 0.01 | 0.11 ± 0.01 |
| **Width (cm)** | 0.15 ± 0.01 | 0.05 ± 0.01 |
| **Region in Algeria** | Thniet El Abed (Batna, Algeria) | |
| **GPS coordinates** | 35°20' N ; 6°20' E | |

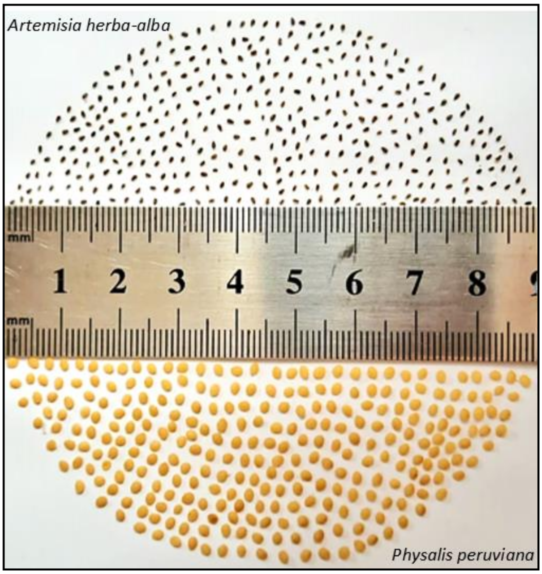


Figure 1. Seeds of Artemisia herba-alba and Physalis peruviana.

**Experimental design and application of salt stress**

The germination test was performed in plastic Petri dishes (9 cm Ø) included one Whatman filter paper moistened of the different saline concentrations (0, 50, 75, 100 and 200 mM) of NaCl (Table 2). For each salinity level, three replicate Petri dishes, each with 50 seeds, were wrapped in aluminum foil (continuous dark) and incubated under 25 °C (±2 °C). Maintaining a specific humidity level for the seeds was a critical aspect of the experiment. The papers were replaced every three days to prevent salt accumulation during the 22 days of the experiment. A complete randomized design was used to conduct the germination test (Kheloufi and Mansouri, 2019).

Table 2. Saline solutions and corresponding pH and electrical conductivity

| Concentrations (mM NaCl) | pH at 19°C | Electrical conductivity (EC) ($mS.cm^{-1}$) at 23.3°C |
|---|---|---|
| **0 (Control)** | 9.11 | 0.03 |
| **50** | 8.80 | 5.08 |
| **75** | 7.81 | 7.55 |
| **100** | 8.09 | 9.52 |
| **200** | 8.04 | 18.7 |

**Germination parameters**

***Final germination percentage (FGP):*** The FGP designates the seeds that successfully germinated relative to the total number of seeds sown in each Petri dish. This parameter was determined using the formula:

$$\text{FGP (\%)} = \frac{\sum \text{ni}}{\text{N}} \times 100$$

where FGP is the final germination percentage, ni is the number of germinated seeds on the last day of the test, and N is the total number of seeds incubated per test (Côme, 1970).

***Mean Germination Time (MGT):*** The MGT indicates the rate at which seeds germinate within a population. A reduced MGT value reflects a faster germination rate, while a higher value signifies a slower rate. MGT was calculated using the following formula:

$$\text{MGT (days)} = \frac{\sum(ti.\,ni)}{\sum ni}$$

where MGT is the mean germination time, ti is the number of days since the beginning of the test, ni is the number of germinated seeds recorded at time t(i), and Σni is the total number of germinated seeds (Orchard, 1977).

***Time to 50% germination ($T_{50}$):*** The $T_{50}$ was designed to determine the time needed for 50% of the seeds to germinate. It is calculated using the following formula:

$$\text{T50 (days)} = \frac{\text{ti} + (\text{N}/2 - \text{ni})(\text{tj} - \text{ti})}{(\text{nj} - \text{ni})}$$

where N final number of seeds emerged, nj and ni are the cumulative numbers of seeds emerged after adjacent counts during tj and ti, when ni < N/2 > Nj (Coolbear et al., 1984).

***Germination Tolerance Index (GTI):*** The GTI is a quantitative parameter used to evaluate the capacity of seeds to germinate under varying salinity levels. The calculation follows the formula provided by Khan and Ungar (1997):

$$\text{GTI (\%)} = \frac{\text{FGP under stress condition}}{\text{FGP under non} - \text{stress condition}} \times 100$$

**Statistical analyses**

The effects of different NaCl concentrations on the four variables studied were tested by a one-way and two-way analysis of variance (ANOVA). Differences between treatments following ANOVA were made by means comparison. Multiple comparisons of means were carried out using Tukey's test ($p \leq 0.05$). A repeated measures analysis of variance was carried out for the germination kinetics. All statistical analyses were performed using SAS software Version 9.0 (Statistical Analysis System) (2002).

## RESULTS AND DISCUSSION

The data presented in Figure 2 displays the overall germination rates for seeds of *Artemisia herba-alba* and *Physalis peruviana* over a period of 22 days as a function of increasing NaCl concentrations (mM). The figure highlights three distinct phases: an initial phase of seed imbibition resulting in a latency period, followed by an exponential phase of rapid germination, and, finally, a plateau phase indicating a cessation in germination (stationary phase). Notably, the two species were able to germinate at all NaCl concentrations during the 22-day experimental period.

For *A. herba-alba*, the control group shows 17.3% germination by the 6$^{th}$ day with a stationary phase starting on the 21$^{st}$ day. Seeds treated with 50, 75, and 100 mM have a low initial germination rate at the 6th day, which improves starting of the 14th day with a stationary phase not exceeding 40% of germination (Figure 2).

For *P. peruviana*, the stationary phase begins on the 6th day in the control and 50 mM NaCl group with 100% germination. As salinity increases, the stationary phase starts at around the 10$^{th}$ day for 75 mM NaCl and 100 mM NaCl and the germination rate decreases with increasing NaCl concentration. As the NaCl concentration increases by 200 mM, the exponential phase begins with a lower germination rate, reaching 6% germination at the 13th day and reaching the maximum at the 21st day with 36.7% germination.

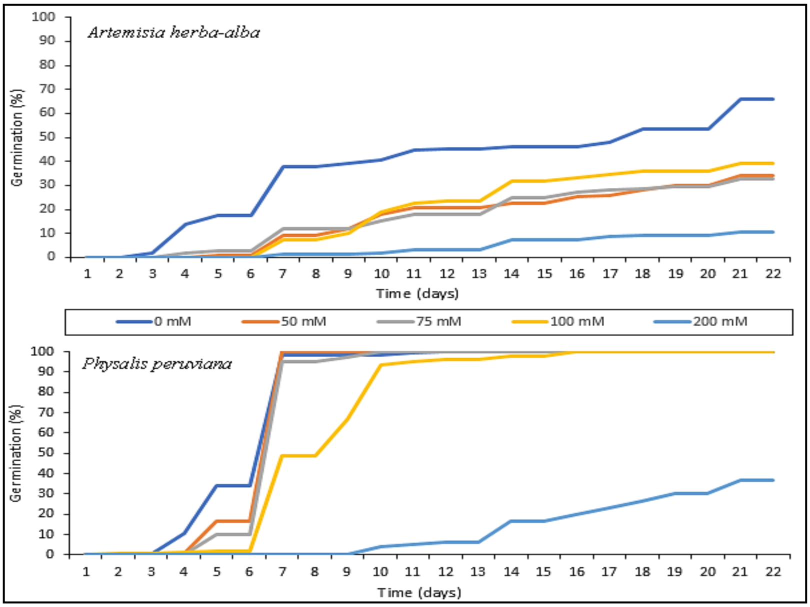


Figure 2. Cumulative germination percentages of *Artemisia herba-alba* and *Physalis peruviana* seeds treated with NaCl for a 22-day period.

According to Figure 2, the germination rates of all the species examined decrease with increasing salinity stress, and NaCl has a significant effect on this reduction especially at 200 mM ($p<0.001$). In addition, it is evident that the length of the latency period varies among species and increases as the concentration of NaCl increases. A repeated measures analysis of variance (performed over a 22-day period with daily evaluations) indicates that there is a highly significant effect ($p<0.001$) between various factors and variables, such as salinity concentration, species, and time, with both between-subject and within-subject effects and their correlation.

The delay in germination and inhibition of growth induced by salinity is caused by various factors such as reduced external water potential, ion imbalance, and specific ion toxicity (Soni *et al.*, 2023). In such conditions, there is a reduction in water uptake alongside an over-absorption of ions. The salinity tolerance of seeds includes both their duration in the soil, during which they may encounter elevated salinity levels and extreme temperatures, as well as their germination phase (Haider *et al.*, 2023).

Table 3. Effect of NaCl concentrations on germination traits of *Artemisia herba-alba* and *Physalis peruviana*

| Species | Salinity (NaCl) | FGP (%) | MGT (days) | $T_{50}$ (days) | GTI (%) |
|---|---|---|---|---|---|
| ***Artemisia herba-alba*** | 0 mM | $67.3^{a}$ | $3.81^{a}$ | $6.01^{ab}$ | $100^{a}$ |
| | 50 mM | $39.3^{b}$ | $4.14^{a}$ | $5.73^{ab}$ | $58.5^{b}$ |
| | 75 mM | $32.7^{b}$ | $3.89^{a}$ | $5.99^{ab}$ | $48.5^{b}$ |
| | 100 mM | $34.1^{b}$ | $4.42^{a}$ | $8.21^{a}$ | $50.9^{b}$ |
| | 200 mM | $10.7^{c}$ | $1.26^{a}$ | $1.91^{b}$ | $16.2^{c}$ |
| | **F-value** | 24.76 | 1.92 | 4.71 | 22.73 |
| | ***p*-value** | <0.001 | 0.183 | 0.021 | <0.001 |
| ***Physalis peruviana*** | 0 mM | $100^{a}$ | $5.61^{a}$ | $5.19^{a}$ | $100^{a}$ |
| | 50 mM | $100^{a}$ | $5.82^{a}$ | $5.39^{a}$ | $100^{a}$ |
| | 75 mM | $100^{a}$ | $5.97^{a}$ | $5.47^{a}$ | $100^{a}$ |
| | 100 mM | $100^{a}$ | $6.51^{a}$ | $6.12^{a}$ | $100^{a}$ |
| | 200 mM | $36.7^{b}$ | $1.45^{b}$ | $2.00^{a}$ | $36.7^{b}$ |
| | **F-value** | 59.77 | 32.34 | 3.26 | 59.77 |
| | ***p*-value** | <0.001 | <0.001 | <0.001 | <0.001 |

FGP-final germination percentage; MGT-mean germination time; $T_{50}$-time to 50% germination; GTI-germination tolerance index.

Table 3 summarizes the effects of varying NaCl concentrations on the final germination percentage (FGP), mean germination time (MGT), time to 50% germination ($T_{50}$), and germination tolerance index (GTI) for *A. herba-alba* and *P. peruviana*, along with the results of a one-way ANOVA for each species. In addition to the results presented, the statistical analysis conducted through a two-way ANOVA provides further insights into the effects of salinity, species, and their interactions on seed germination parameters (Table 4).

*A. herba-alba* exhibited varying responses to increasing NaCl concentrations. At lower concentrations (0 mM and 50 mM), the FGP was relatively high, with values of 67.3% and 39.3% respectively. However, as salinity levels increased to 75 mM and 100 mM, the FGP declined substantially to 32.7% and 34.1% respectively. Notably, the highest NaCl concentration (200 mM) resulted in a significant reduction in FGP to 10.7%, indicating a pronounced inhibitory effect on germination (Table 3). These findings suggest that *A. herba-alba* is moderately sensitive to salinity stress during seed germination, with higher concentrations exerting greater inhibitory effects. Similar findings were reported by Nedjimi and Zemmiri (2019), demonstrating a significant decrease in final germination percentage (FGP) with increasing salinity levels. The highest FGP of 80% was observed in the distilled water control group. Salinity can influence germination by promoting the uptake of toxic ions, which in turn can lead to alterations in certain enzymatic or hormonal activities within the seed (Martínez-Ballesta *et al*., 2020). Salinity has been reported to cause substantial reductions in both the rate and final percentage of germination and emergence across various vegetable crops. Consequently, this may lead to uneven stand establishment and reduced crop yields (Gul *et al.,* 2022).

In terms of germination timing, *A. herba-alba* seeds exposed to different NaCl concentrations exhibited comparable MGT values, ranging between 3.81 to 4.42 days. However, the time to 50% germination (T50) showed slight variations across treatments, with seeds exposed to 200 mM NaCl requiring significantly less time (1.91 days) compared to other concentrations. This accelerated germination rate at higher salinity levels may be attributed to osmotic adjustment mechanisms triggered by salt stress, aiming to mitigate the adverse effects on seedling establishment.

The germination tolerance index (GTI) provides a comprehensive measure of seedling performance under salinity stress, considering both germination percentage and germination timing. *A. herba-alba* seeds exhibited the highest GTI (58.5%) when exposed to 50 mM NaCl, indicating relatively better tolerance to moderate salinity levels. However, as salinity increased, the GTI declined progressively, reaching the lowest value of 16.2% at 200 mM NaCl (Table 3). This decline in GTI underscores the detrimental impact of high salinity on seedling vigor and overall germination performance in *A. herba-alba.*

In contrast, *P. peruviana* demonstrated remarkable tolerance to salinity stress during seed germination. *P. peruviana* seeds consistently achieved a high FGP of 100%, indicating minimal inhibitory effects on germination even at elevated salinity levels. This high germination percentage suggests intrinsic physiological adaptations that enable *P. peruviana* seeds to tolerate salt stress during germination, thus ensuring successful establishment under adverse environmental conditions. Furthermore, both MGT and $T_{50}$ values remained relatively consistent across different NaCl concentrations for *P. peruviana*, indicating that salinity did not significantly influence the timing of germination. This consistent germination timing suggests efficient physiological processes involved in seed imbibition and embryo development, unaffected by salt stress (Dey and Bhattacharjee, 2023).

The GTI values for *P. peruviana* remained consistently high across all salinity treatments, maintaining optimal seedling performance irrespective of NaCl concentration. This remarkable germination tolerance underscores the species' resilience to salinity stress during the critical germination stage, highlighting its potential for cultivation in salt-affected soils. Several authors have described a decrease in germination attributed to elevated salinity levels (Alkharabsheh *et al*., 2021). The present study revealed significant differences in all observations concerning salinity. These findings are consistent with earlier observations made for several cultivars of golden berry (Miranda *et al*., 2010; Yildirim *et al*., 2011; Cebeci and Hanci, 2015).

For FGP, both salinity (F=67.95, p<0.001) and species (F=424.97, p<0.001) showed significant main effects, indicating their individual contributions to variations in germination percentage. Additionally, the interaction between salinity and species (S×SP) was also significant (F=12.86, p<0.001), suggesting that the effects of salinity on FGP varied between *A. herba-alba* and *P. peruviana*.

Table 4. Two-way ANOVA of salinity and species effects on germination traits of *Artemisia herba-alba* and *Physalis peruviana*.

| **Variables** | **Factors** | **df** | **Mean square** | **F-value** | ***p*-value** |
|---|---|---|---|---|---|
| **FGP** | S | 4 | 3062.13 | 67.95 | <0.001 |
| | SP | 1 | 19152.13 | 424.97 | <0.001 |
| | S × SP | 4 | 579.46 | 12.86 | <0.001 |
| **MGT** | S | 4 | 16.56 | 11.29 | <0.001 |
| | SP | 1 | 18.39 | 12.53 | <0.001 |
| | S × SP | 4 | 0.93 | 0.64 | ns |
| **$T_{50}$** | S | 4 | 22.51 | 7.84 | <0.001 |
| | SP | 1 | 4.05 | 1.41 | ns |
| | S × SP | 4 | 1.02 | 0.36 | ns |
| **GTI** | S | 4 | 4391.15 | 55.16 | <0.001 |
| | SP | 1 | 7933.82 | 99.66 | <0.001 |
| | S × SP | 4 | 719.41 | 9.04 | <0.001 |

FGP-final germination percentage; MGT-mean germination time; $T_{50}$-time to 50% germination; GTI-germination tolerance index; S-salinity; SP-species; df-degree of freedom; ns-non significant at p<0.05.

Similarly, MGT exhibited significant main effects of both salinity (F=11.29, p<0.001) and species (F=12.53, p<0.001), indicating their influence on the timing of germination. However, the interaction between salinity and species was not significant (p>0.05), suggesting that the effect of salinity on MGT did not differ significantly between the two species. For $T_{50}$, salinity demonstrated a significant main effect (F=7.84, p<0.001), indicating its impact on the time required for 50% germination. However, the effect of species and the interaction between salinity and species were not significant (p>0.05), suggesting that both *A. herba-alba* and *P. peruviana* responded similarly to salinity in terms of $T_{50}$. Regarding GTI, significant main effects of salinity (F=55.16, p<0.001) and species (F=99.66, p<0.001) were observed, indicating their influence on seedling vigor under different salinity levels. Additionally, the interaction between salinity and species (S×SP) was significant (F=9.04, p<0.001), suggesting differential responses of *A. herba-alba* and *P. peruviana* to salinity stress in terms of GTI (Table 4).

Despite the harmful effects of NaCl, this study shows that seeds of *A. herba-alba* and *P. peruviana* can germinate under 200 mM (Table 3). Such a salt concentration is considered to correspond to a significantly high level of salinity. This level of salinity exceeds the salinity tolerance level of the majority of cultivated vegetable species, as well as several halophytes (Bayuelo-*Jiménez et al.*, 2002; Malcolm *et al.*, 2003). As noted by Ungar (1982) and more recently by Suleiman *et al*. (2023), seeds of many perennial species possess the ability to preserve their viability for prolonged periods of exposure to harsh conditions, especially salinity and drought, and then to propagate when the ecological conditions are favorable. The maturation of seeds common to arid climates takes place during the autumn and the seeds begin to germinate within a few days of the first precipitation of the spring season. The seeds are typically found in the surface layers of the soil and propagate when high salt concentrations are leached away by rainfall.

## CONCLUSIONS

Contrasting responses of *A. herba-alba* and *P. peruviana* to salinity stress during seed germination highlight species-specific adaptive mechanisms influencing germination performance under adverse conditions. Our analysis of germination attributes and statistical assessments reveals species-specific reactions to varying NaCl concentrations, offering valuable insights into their adaptive strategies under saline conditions. *A. herba-alba* showed moderate sensitivity to salinity, with decreasing germination percentages and tolerance indices as NaCl levels increase. In contrast, *P. peruviana* displayed remarkable resilience, maintaining high germination rates and vigorous tolerance across all salinity treatments. These distinct responses underscore the importance of species-specific adaptations in mitigating salinity stress effects on seed germination. These findings develop our knowledge of seed responses to salinity stress, relevant for conservation, cultivation, and management of these economically and ecologically vital species in saline-affected areas. Further exploration of molecular and physiological mechanisms behind salinity tolerance is essential for developing resilient crop varieties and sustainable agricultural practices in saline environments. Cultivating these species for horticultural purposes supports biodiversity while providing effective strategies to enhance agricultural productivity in challenging environments.

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# EFFECT OF BIOSTIMULATOR ON YIELD COMPONENTS AND CRUDE PROTEIN CONTENT OF THE POPULATION OF WHITE LUPINE (*Lupinus albus* L.)

## SUMMARY

Experimental studies of the impact of biostimulators on yield components, grain yield and crude protein content in white lupine grains were conducted in the period 2020-2022, in two variants: V1 - Control, variant without biostimulator and V2 - variant with biostimulator in the Agricultural Institute of the Republic of Srpska, Banja Luka. The goal of the research was to examine the effectiveness of the applied biostimulator on the analyzed properties of white lupine under production conditions. The sowing of lupine, in all years, was done by hand during March. The following parameters were analyzed: number of pods per plant, number of grains per pod, weight of grains per pod (g), weight of 1000 grains (g), grain yield (kg ha$^{-1}$), crude protein content in grains (g kg$^{-1}$) and alkaloid content in grains (%). The number of pods per plant in the control variant was on average 5.0, and the pods contained 3.0 grains, while in the V2 variant it was 7.8 with 3.6 grains. The average weight of 1000 grains in the control was 221.2-254.1 g, and in the V2 variant 259.5-279.3 g. The grain yield in the control was 1386.8 kg ha$^{-1}$, and in the biostimulator treatment 1806.6 kg ha$^{-1}$. Crude

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protein content varied from 254.0 g $kg^{-1}$ DM in the control treatment to 279.8 g $kg^{-1}$ DM in the variant with biostimulator. The white lupine population tested in the experiment had a high alkaloid content and is not suitable for animal and human consumption, but can be grown for green manure.

**Keywords**: white lupine, biostimulator, pods, grain yield, crude protein

## INTRODUCTION

*Lupinus albus* L. - white lupine is an annual legume that can be grown for above-ground production of biomass or grains, as cover crops, for biological suppression of soil parasites and remediation of degraded lands (Fumagalli *et al*., 2014). Nitrogen-fixing bacteria *Bradyrhizobium lupini* live on the roots of white lupine, which allows up to 240 kg $ha^{-1}$ of nitrogen to accumulate in the soil during the growing season (Mateos *et al*., 2015). The characteristic of this plant species is that can absorb hardly soluble phosphates from the soil. Lupine populations that have a high content of toxic alkaloids are suitable for growing on soils that are infected with nematodes because in this way these pathogenic organisms can be suppressed. The produced biomass of white lupine with a high alkaloid content in areas infected with nematodes is used as green manure. The cultivation of white lupine affects the restoration of the general condition of poor soils with a heavier mechanical composition. Thanks to its powerful root system, it can draw water from the deeper layers of the soil. According to the soil, lupins are not demanding. They thrive on different types of soil, from sandy to heavy clay soils (Gresta *et al*., 2017). Alkaline soils cause chlorosis and reduce growth, although some cultivars are more tolerant of soil salinity and heavy soils (Lim, 2012). Lupins are tolerant of growing in monoculture and can make better use of more difficult-to-access plant nutrients from acidic soils.

Producers use a variety of organic, mineral, and microbial fertilizers to attain the proper high yields (Božović *et al.,* 2018; Ugrenović *et al.,* 2021; Govedarica-Lučić *et al.,* 2020). The yield and quality of legumes depend mostly on balanced mineral nutrition. The quality of the yield can also be seen by the ratio of crude protein and crude fiber (Enchev and Bozhanska, 2024). The mineral nutrition of legumes depends on the type of soil, climatic factors of the region, and other agroecological factors (Popović *et al*., 2020; Popović *et al*., 2022; Kosev *et al*., 2022; Vasileva *et al.,* 2023; Karthika *et al*., 2023). Biostimulators are plant extracts that contain a wide range of bioactive compounds capable of improving certain physiological processes that stimulate the growth and development of crops (Bulgari *et al*., 2015). The use of biostimulators is one of the promising alternative methods and solutions for improving the production of legumes (Parihar *et al.*, 2022). Biostimulators in the soil affect the microflora and thus can positively influence the growth and development of plants (Kunicki *et al.*, 2010). By applying biostimulator on lupine grains before sowing, it stimulates the processes of germination and sprouting of plants, and has a positive effect on the development of the root system and aerial parts of cultivated plants. The positive influence of seed treatment with the microbiological biostimulator on the yield of small-grained broad beans is reported by Lakić *et al*. (2022). The goal of the research was to test the effectiveness of the applied biostimulator on the analyzed features of white lupine under production conditions.

## MATERIAL AND METHODS

*Field experiment*

The study of the impact of biostimulators on grain yield and crude protein content in white lupine grains was carried out in the period from 2020-2022. Field research was carried out on the experimental field of the Agricultural Institute of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina. The seeds of the autochthonous population of white lupine were used, taken from the gene bank of the Institute of Genetic Resources, University of Banja Luka. During 2018, the alkaloid content in the white lupine seeds was determined. The sowing rate was 160 kg $ha^{-1}$. A two-factorial experiment (factor A – treatment, factor B – year) was set up in four replicates. The size of the basic sample plot was 10 $m^2$. The distance between the rows was 50 cm, and in the row was 8 cm. No mineral fertilizers were used during these tests. Sowing of white lupine, in all years, was done mechanically, during March. White lupine seeds were treated before sowing with the microbiological biostimulator, fertilizer containing: nitrogen fixers - bacteria that perform the process of nitrogen fixation; phosphomineralizators - bacteria that break down organic phosphorus compounds (which make up 50-80 % of phosphorus in the soil) and convert them into an accessible form ($P_2O_5$) and growth regulators - auxins, which contains indole-3 acetic acid.

During these researches, the following parameters were analyzed: content and type of alkaloids in the grain (%), number of pods per plant, number of grains per pod, weight of grains per pod (g), 1000 grains mass (g), grain yield (kg $ha^{-1}$) and crude protein content in grain (g $kg^{-1}$). The alkaloid content of white lupine seeds was determined using Kapillar-GLC at the Institute für Pharmazie und Molekulare Biotechnologie, Ruprecht-Karls-Universität Heidelberg.

Thirty white lupine plants were counted and measured in each repetition for each treatment in order to ascertain the studied attributes, number of pods per plant, number of grains per pod, and weight of grains per pod.

The weight of 1000 grains (g) was determined from the pure seed fraction in the Laboratory for Seed Quality Control, Agricultural Institute of the Republic of Srpska in Banja Luka. With a seed counter, 100 seeds were sampled in 8 repetitions (ISTA Rules, 2018).

The yield of white lupine seeds was determined at the stage of full maturity per replicates, separately for each treatment, and then converted to a unit area. The white lupine harvest was performed in the first half of the seventh month.

The content of crude proteins in white lupine grains was determined according to the Kjeldahl method - BAS EN ISO 5983-2:2010.

*Statistical analysis*

Experimental data were analysed by descriptive and analytical statistics using the statistics module Statistica 12. All evaluations of significance were made on the basis of the ANOVA test at 0.05% and 0.01% significance levels. In cases where a significant difference was found, the LSD test was used. The significance of the differences was established at the level of $p<0.05$ for significant differences and $p<0.01$ for highly significant differences.

*Soil analysis*

The experiment was performed on the soil type determined as valley-brown soil on the alluvial substrate. Before setting up the experiment, soil samples were taken from the arable layer from a depth of up to 30 cm, and the results of the chemical analyzes are shown in Table 1. The pH value of the soil was neutral, the hummus content was average, the provision of soil with easily accessible phosphorus (10.2 mg/100) was moderate, and easily accessible potassium (30.6 mg/100) was very good. Based on the results of the chemical analysis of the soil sample the tested soil is suitable for growing white lupine.

Table 1. Agrochemical properties of soil

| Depth (cm) | pH in KCl | pH in $H_2O$ | Humus (%) | $NH_4$ (%) | $NO_2$ (%) | $P_2O_5$ mg/100 g | $K_2O$ mg/100 g |
|---|---|---|---|---|---|---|---|
| 0-30 | 5.8 | 6.8 | 3.2 | 1.5 | 3.3 | 10.2 | 30.6 |

*Meteorological data*

The analysis of mean monthly temperature (ºC) and sum of monthly precipitations (mm) was performed based on data obtained from the Republic Hydrometeorological Institute in Banja Luka. The analyzed climate elements were compared with the multi-year average for the Banja Luka area. Average monthly air temperatures and total precipitation by month for the period 2020-2022 and the ten-year average (2011-2020) are shown in Figures 1-4.

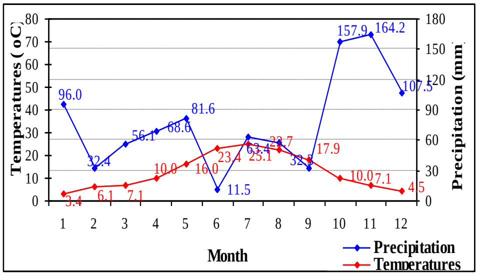


Figure 1. Temperatures (°C) and precipitation (mm) in Banja Luka, B&H (2020)

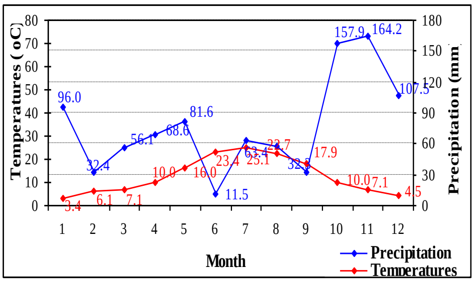


Figure 2. Temperatures (°C) and precipitation (mm) in Banja Luka, B&H (2021)

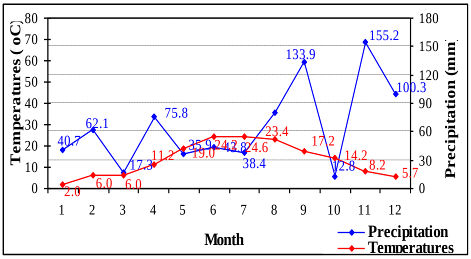


Figure 3. Temperatures (°C) and precipitation (mm) in Banja Luka, B&H (2022)

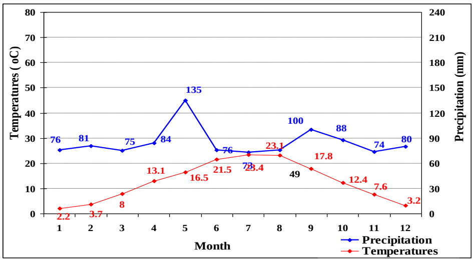


Figure 4. Temperatures (°C) and precipitation (mm) long-term average, Banja Luka, B&H (2011-2020)

In 2020, the amount of precipitation during the growing season was 322 mm, which is 121 mm less than the multi-year average. The maximum amount of precipitation was recorded in May (Figure 1).

Average monthly temperatures during the growing season in 2020 ranged from 7.8 ºC to 22.3 ºC in July. The average monthly temperature was lower than the ten-year average (Figure 4).

During the second year of the research, the amount of precipitation during the growing season (III-VII) of white lupine was lower compared to the previous multi-year period. The maximum amount of precipitation was recorded in April. The precipitation schedule in 2021 was less favorable (Figure 2). A dry period began in mid-May.

During 2021, the monthly temperatures during the growing season ranged from 6.1 ºC to 25.1 ºC in July (Figure 2).

In the period from March to the end of June 2022, a significantly lower amount of precipitation fell compared to the ten-year average from 2011-2020. The highest amount of precipitation during the growing season was in April (Figure 3).

Temperatures varied from 6.0 ºC in March to 24.6 ºC in July. The average monthly temperature during the growing season was about 2.5 ºC higher than the annual average (Figure 4).

During the duration of these studies, the most favorable conditions for growing white lupine were in 2020, and the worst in 2021 due to the dry period in the second part of the growing season.

## RESULTS AND DISCUSSION

*Yield components and crude protein content*

The results of three-year tests on the impact of biostimulators on yield components and crude protein content in white lupine grains are shown in table 2.

The number of pods per plant in the control variant was an average of 5.0, and the pods contained 3.0 grains. In the V2 variant on the white lupine plants, there were an average of 7.8 pods, and in them there were an average of 3.6

grains. The V2 variant resulted in a higher number of pods/plant compared to the control during tested three years, Tab. 2.

Table 2. Average values for tested morpho-productive traits of tested genotypes, 2020-2022

| Variant | Year | Number of pods/ plant – NPP | No of grains/ pod - NGP | Grain mass /pod- GMP (g) | 1000 grains mass - TGM (g) | Grain yield - GY (kg ha$^{-1}$) | Protein content - PC (g kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Control | 2020 | 6.0 | 3.3 | 1.09 | 225.8 | 1 628.5 | 256.6 |
| | 2021 | 4.3 | 2.8 | 1.04 | 221.2 | 1 095.1 | 257.1 |
| | 2022 | 4.8 | 3.0 | 0.99 | 254.1 | 1 436.8 | 248.3 |
| | Average | 5.0 | 3.0 | 1.04 | 233.7 | 1 386.8 | 254.0 |
| Biosti-mulator | 2020 | 9.5 | 3.8 | 0.93 | 278.4 | 2 089.8 | 280.4 |
| | 2021 | 6.8 | 3.5 | 0.94 | 259.5 | 1 396.9 | 282.5 |
| | 2022 | 7.0 | 3.5 | 1.01 | 279.3 | 1 933.1 | 276.6 |
| | Average | 7.8 | 3.6 | 0.96 | 272.4 | 1 806.6 | 279.8 |

Table 3. Results of the provided analysis of variance

| Parameter | LSD | G - Genotype | Y- Year | G x Y |
|---|---|---|---|---|
| NPP | 0.05 | 0.92* | 1.12* | 1.59 |
| | 0.01 | 1.25** | 1.54** | 2.17 |
| NGP | 0.05 | 0.70 | 0.86 | 1.21 |
| | 0.01 | 0.96 | 1.17 | 1.66 |
| GMP | 0.05 | 0.08 | 0.10 | 0.15 |
| | 0.01 | 0.12 | 0.14 | 0.20 |
| TGM | 0.05 | 6.51* | 7.97* | 11.28* |
| | 0.01 | 8.92** | 10.92** | 15.45** |
| GY | 0.05 | 122.02* | 149.45* | 211.35 |
| | 0.01 | 167.15** | 204.72** | 289.51 |
| PC | 0.05 | 0.70* | 0.86 | 1.21 |
| | 0.01 | 0.96** | 1.17 | 1.66 |

A greater number of grains/pod, during the duration of these tests, was achieved in the V2 variant compared to the control. The influence of the biostimulator on the number of pods/plant was statistically highly significant. The average number of pods/plant in the white lupine Energy variety was 3.9-5.4, while in the Arabella variety it varied from 5.4-8.9 (Pospišil and Pospišil, 2015). According to Šatović (1992), the number of grains in a pod of white lupine varied from 2.4‑5.2. During the tests conducted by Gresta *at al*. (2023) the number of grains in a pod of white lupine was 3.5 on average, and the lowest number of grains was in the variety Volos (2.4). According to Mahfouze *et al.* (2018) during two-year trials the number of pods/plants varied from 3.67-12.0, and the number of grains/pods ranged from 3.66±0.33 - 5.67±0.32. The results of the mentioned authors are in agreement with the results we reached during these researches.

The mass of grains/pod, during testing years, was higher in the control. The average mass grains/pod in the control was 0.99-1.09 g, and in the V2 variant it varied from 0.93-1.01 g (Tab. 2).

During all years the 1000 grains mass was higher in the V2 variant compared to the control. In the control, the 1000 grains mass was 221.2-254.1 g, that is, on average 233.7 g. The 1000 grains mass in the V2 variant was 272.4 g, that is, it varied from 259.5-279.3 g. During these tests, variant, year, and variant x year interaction had a statistically highly significant effect on the 1000 grains mass (Tab. 3). According to the results of Pospišil and Pospišil (2015), the 1000 grains mass in the Energy variety was 290.7-378.9g. Šatović (1992) states that the 1000 grains mass can vary from 164-537 g. According to research by Gresta *et al.* (2023), the 1000 grains mass of white lupine was 426.3 g and 326.7 g on average. The average three-year yield in the control was 1386.8 kg ha$^{-1}$, that is, it varied from 1095.1 to 1628.5 kg ha$^{-1}$. In the biostimulator variant, the grain yield varied from 1396.9-2089.8 kg ha$^{-1}$, that is, on average, it was 1806.6 kg ha$^{-1}$. In these tests, a statistically highly significant effect of variant and year on the yield was determined. The achieved average yields of white lupine grains were from 1293 to 2591 kg ha$^{-1}$ (Lakić *et al.*, 2018). The average grains yield varied depending on the year from 2130-3033 kg ha$^{-1}$ (Pospišil and Pospišil, 2015).

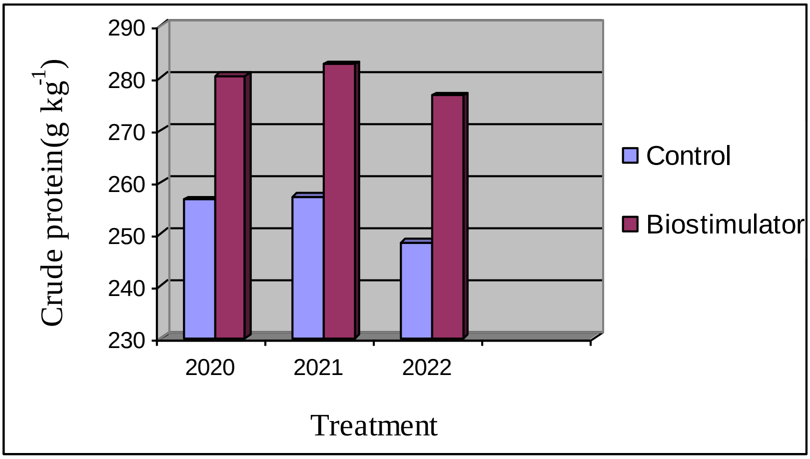


Figure 5. Crude protein content (g kg$^{-1}$) in the grains of white lupine, 2020- 2022

In the control, the average crude proteins content in white lupine grains was 254.0 g kg$^{-1}$ DM, while in the V2 variant it was 279.8 g kg$^{-1}$ DM, Figure 5. The highest crude protein content was determined in 2021 in V2 variant and is 282.5 g kg$^{-1}$ DM or 28.25% of crude proteins. Treatment with biostimulator had a statistically highly significant effect on the content of crude proteins (Table 3). According to the results of Georgieva *et al.* (2018) crude protein content in white lupine grains ranged from 24.90% to 33.30%.

*Alkaloid content*

During these studies, the content of alkaloids in white lupine seeds was tested. The tested population of white lupine contains up to 2.5% alkaloids and belongs to bitter lupine. Lupanin was the most abundant alkaloid in lupine seeds, and secondary alkaloids were 13-Hydroxylupanin, Isolupanin, Multiflorin, Albin, Angustifolin, 13-Tigloyloxylupanin. According to research by Boschin *et al.* (2008), the alkaloid content of the Luxor (6.1 mg 100 $g^{-1}$) and Volos (5.2 mg 100 $g^{-1}$) varieties is much lower than the toxicity threshold (20 mg 100 $g^{-1}$) for human or animal consumption as announced by the health authorities of Australia, France, and the United Kingdom.

Gresta *at al.* (2023) state that the alkaloid content in the varieties Volos, Luxor and Lublanc is significantly lower (0.05–0.19 g $kg^{-1}$) compared to the variety Multitalia and local ecotypes of white lupine (2.0‑2.5 g $kg^{-1}$). Alkaloids are toxic to both humans and animals (Vishnyakova *et al*., 2020), so two general thresholds for the presence of alkaloids in food derived from lupine have been established, namely: 0.02% of dry matter for animal feed and 0.01% of dry matter for human consumption (Otterbach *et al*., 2019).

## CONCLUSIONS

Treatment with biostimulator and the year influenced the increase in the number of pods per white lupine plant. The number of pods on lupine plants grown with the biostimulator treatment was on average 2.8 higher than the control. The effect of variant and year was not influenced on the number of grains/pods and weight of grains/pods. The 1000 grains mass, during all tested years, was higher in the treatment with the biostimulator compared to the control. Year and the interaction effect of treatment x year had a highly significant influence on this feature. The effect of the changed treatment with the biostimulator on the grain yield of white lupine, during all three years of testing, was highly significant. The grain yield in the treatment with the biostimulator was 419.8 kg higher in the three-year harvest compared to the control. During all three years of research, a highly significant influence of the biostimulator on the content of crude nutrients in white lupine grains was determined. The white lupine population tested in the experiment has a high alkaloid content and is classified as bitter lupine. It is not suitable to be used for animal and human consumption, but it can be grown for green fertilization.

## AUTHORS’ CONTRIBUTIONS

All authors have participated in this research. ŽL has designed, supervised and written the paper; VP, VR, MA, DM, RD and WG have participated in the experimentation and sample collection; Conceptualization: VP, VR, DM and AM have analyzed the data obtained; VP and VR have overseen the project and revised the manuscript. All authors have approved the final manuscript.

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# GROWTH PATTERN OF GOAT KIDS FROM BIRTH TO WEANING PERIOD OF TWO IMPORTED BREEDS REARED IN SOUTHEAST ALBANIA

## SUMMARY

This study aimed to evaluate the growth parameters from birth to weaning of Alpine and Saanen goat kids and determine the influence of factors like litter size and sex. The study was conducted on the farm of the Center of Agricultural Technology Transfer of Korca region located in the SothEast of Albania. The data were collected from a total of 50 goat kids, monitoring the body weight at different ages, types of birth, and sexes. The obtained results showed that Alpine goat kids weighed 3.59 kg at birth, 7.85 kg at 30 days and 13.01 kg at 60 days. At birth, Saanen goat kids' weight was 3.50 kg, at 30 days 7.66 kg, and at 60 days 12.43 kg. The differences between these two breeds for three weighed ages were not statistically significant ($p>0.05$). A statistically significant relationship ($p<0.01$) was observed between the type of birth and birth weight. Single goat kids tended to have greater weights than doubles and triples kids. Sex showed a significant influence on birth weight for Alpine goat kids. Females tended to be lighter compared to males throughout the study. Average daily gains from birth to 60 days of goat kids were 0.157 kg and 0.148 kg for Alpine and Saanen, respectively. Both Alpine and Saanen goat kids realized a satisfactory growth performance.

**Keywords**: goat kids, body weight, litter size, breed, average daily gain

## INTRODUCTION

Goat farming is a primary agricultural activity, especially in the mountainous and hilly regions of Albania. In 2020, the total goat population was 774 thousand, while the number of milked goats reached 1619 thousand

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(INSTAT, 2020). The total goat milk production for the same year amounted to 80 thousand liters. Native goat breeds account for approximately 97% of the goat population (Jani and Kume, 2018).

The predominant rearing system is extensive, relying heavily on pasture and grazing throughout most of the year. Animals are only kept and fed indoors during rainy or snowy days. Native goat breeds, well-adapted to harsh environmental conditions, serve as an important source of milk and meat. They play a vital economic role in the lives of farmers, contributing significantly to the local community. Albanian goat farming is characterized by small farms that utilize extensive or semi-extensive management systems. These systems primarily depend on natural grazing with minimal supplemental feed, producing milk and meat mainly for family consumption.

Due to the absence of controlled breeding programs and herd books, local goat breeds exhibit significant genetic admixture and low differentiation (Hoda *et al.*, 2023). Being local breeds, their production levels for both milk and meat are relatively low. However, productivity could be enhanced through genetic selection and improved breeding systems (Răducuţă *et al*., 2007).

To address production challenges, exotic goat breeds such as Alpine and Saanen have been introduced to improve milk and meat yields. These breeds are either breed pure or used for crossbreeding with local goats to enhance productivity. The Agricultural Technology Transfer Center (ATTC) manages herds of 30 Alpine and 50 Saanen goats. These animals serve as purebred nuclei and are used for the genetic improvement of the local goat population (Hoda *et al.*, 2022).

The Alpine goat breed, widely distributed and highly versatile, performs well in both purebred and crossbred forms for milk and meat production (Xhemo *et al.*, 2013). Originating from temperate mountain ranges, Alpine goats are well-suited to cold and heat and are highly adaptable to steep mountain slopes. Known for their even temperament and robust health, Alpine goats produce high quantities of milk, necessitating close monitoring of their diets to meet nutritional needs.

Similarly, the Saanen goat is a renowned dairy breed with great potential as a milk-producing livestock species (Zurriyati *et al*., 2011). Goat milk has advantages over cow’s milk, being easier to digest due to its smaller and more homogeneous fat globules (Jenness, 1980). However, Saanen goats require proper farming conditions, including adequate shade and supplemental nutrition, as they are vulnerable under suboptimal conditions. Crossbreeding programs have shown success when these conditions are met, particularly for purebred exotics and backcrosses beyond the F1 generation.

Improving milk and meat production in small ruminants can also be achieved through the implementation of advanced rearing technologies and the incorporation of supplementary feeding practices. (UNDP, Albania, 2007, Hoda *et al.*, 2009).

This study aimed to evaluate the growth performance of Alpine and Saanen goat kids from birth to 60 days (weaning period) under semi-intensive conditions in southeastern Albania. It also investigated the effects of factors such as litter size and sex on measurable growth criteria, including weight at specific ages and average daily gain (ADG) (Aissaoui *et al*., 2019). Understanding the growth pattern during the early life stage is essential for establishing appropriate feeding practices and determining optimal weaning times based on weight rather than age. Growth data can further support breeding programs designed to enhance not only growth performance but also overall productivity. Early-life growth significantly impacts post-weaning survival and adaptability to rearing conditions.

The ATTC of Korça plays a crucial role in designing programs for goat breed improvement, aligning with market and societal needs to increase livestock production and farmers' incomes (Leka, 2019). Studying the growth capacities of goat kids is key to optimizing management practices and improving the efficiency of the birth-to-weaning period.

## MATERIAL AND METHODS

The current study was performed on two imported goat breeds, “Alpine” and “Sannen” that are reared at the Agricultural Technology Transfer Center (ATTC), located in the SouthEast region of Albania. The records of the body weight of 50 goat kids were collected. Data were recorded from 27 individuals from Alpine breed and 23 from Saanen breed. For each goat kids were recorded type of birth (single, double or triple) and sex (male or female). The weighting time of each animal was performed each month from birth till weaning time (60 days). The weighting was done with electronic balance with an accuracy of 0.01 kg.

The data were analyzed by software package IBM SPSS Statistics 20 and the XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France, 2017). Daily weight gain and weight gain at weaning were calculated. The statistical difference was reported at ($P<0.05$). The generalized linear model (GLM) was used to test the effects of factors on the variables, by applying the independent sample T test and ANOVA to estimate the significance of homogeneity between different sets of data (comparison test between the averages). The correlation results were calculated by the Pearson test for the evaluation of the relationship between live weights at different growth phases.

The growth evolution was estimated using the following growth indices:

- Growth rate: absolute (A) and relative(R);
- Growth intensity (I);
- Growth factor (F);

Growth rate expressed as absolute and relative values, indicates the average body mass increase observed in the animal between determinations (1) and (2). The increase in body mass over a certain period (t) is growth intensity (3). The growth factor is the mass achieved in a given growing period (Mt) of the final animal mass (Mf) expressed as a percentage (Dărăban, 2006).

A= $M2-M1/t$ (1)
R = $M2-M1/M1$ *100 (2)
I = $M2-M1/M2+M1$*2*100 (3)
F = $Mt*$100/Mf (4)

Where:
M1=body mass at t1 (kg); M2=body mass at t2 (kg); Mt=body mass accumulation in a period (kg); Mf=final body mass (kg); t=period between t1 and t2 (days).

## RESULTS AND DISCUSSION

Alpine and Saanen goat kids used in this study were raised at the same farm in the Agricultural Technology Transfer Center (ATTC), so they benefited from the same climatic conditions and feeding. Three birth types, were identified single birth, twin birth, and triple birth (Figure 1).

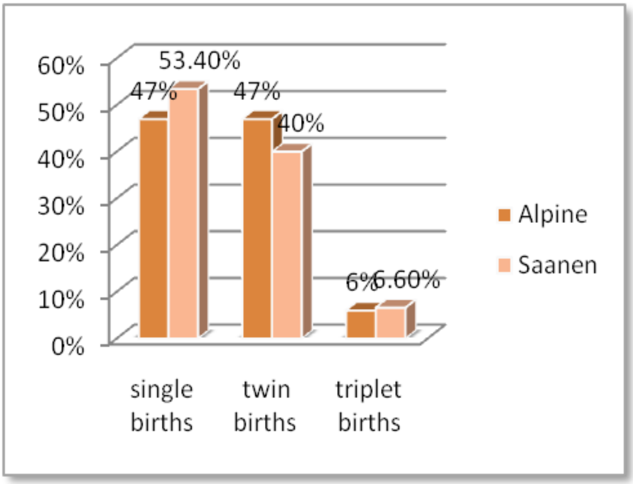


Figure 1. Number of single, twin, triplet births (%) for Alpine and Saanen goat

Alpine goats have the same number of single and twin birth (47%), followed by a low percentage of triple births (6%). Saanen goats have a small difference between single and double births, where the predominant are the single births (53.4%).

The high percentage of female kids number represents an advantage for the farm because the selection can be made much more rigorously, but also males obtained annually have an economic advantage for the farm because they accumulate body mass in relatively less time than females (Marina *et al.*, 2020). The number of female kids for Alpine breed reported in this study was higher than for males, the opposite is for Saanen breed where the predominant are males (Figure 2).

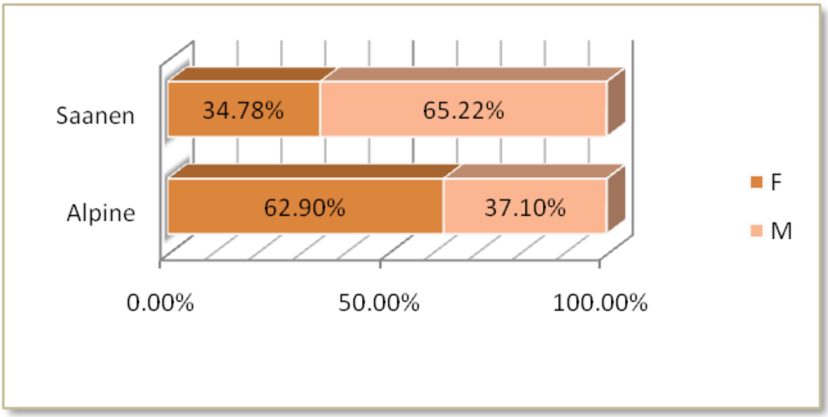


Figure 2. Percentage ratio of females (F) and males (M) for Alpine and Saanen goat kids

Table 1. Descriptive statistics for body weight at birth, at 30 days and 60 days

| Breed | | Weight at birth (kg) | Weight at 30 days (kg) | Weight at 60 days (kg) | Gain weight at 60 days (kg) | Average daily gain (kg) |
|---|---|---|---|---|---|---|
| Alpine N=27 | Mean | 3.59 | 7.85 | 13.01 | 9.42 | 0.157 |
| | SD | 0.605 | 1.186 | 2.701 | 2.463 | 0.041 |
| | SE | 0.116 | 0.228 | 0.519 | 0.474 | 0.007 |
| | Min | 2.00 | 6.00 | 8.00 | 6.00 | 0.10 |
| | Max | 4.50 | 10.30 | 19.00 | 15.00 | 0.25 |
| | V | 0.366 | 1.407 | 7.298 | 6.071 | 0.002 |
| Saanen N=23 | Mean | 3.50 | 7.66 | 12.43 | 8.93 | 0.148 |
| | SD | 0.564 | 1.141 | 2.096 | 1.949 | 0.032 |
| | SE | 0.117 | 0.238 | 0.437 | 0.406 | 0.006 |
| | Min | 2.50 | 6.00 | 8.00 | 5.00 | 0.080 |
| | Max | 4.50 | 10.00 | 16.00 | 13.00 | 0.221 |
| | V | 0.318 | 1.303 | 4.393 | 3.802 | 0.001 |

The birth weight of Alpine goat kids ranged from 2.00 to 4.50 kg with an average value of 3.59±0.605 kg (Table 1). The average birth weight of Saanen goat kids was 3.50±0.564 kg which is slightly lower (0.09 kg) than the Alpine breed. It ranges from 2.50 to 4.50 kg. The differences between these two breeds were not significant (F=0.309, p=0.581).The average birth weight of kids recorded in this study is similar with those obtained from Doize *et al*, for Alpine and Saanen breeds, which recorded birth weights of (3.62 and 3.43 kg); respectively (Doizé *et al*., 2013). However, it was higher than the birth weight recorded in Sahel kids in Senegal (2.24 kg) (Djakba, 2007) the Creole goats of Guadeloupe (1.64 kg) (Chemineau and Grude, 1985), as well asin goats of Maradi breed in Niger (1.85kg) (Djıbrıllou, 1986).

Birth weight is a good indicator of mothers' diets in the last weeks of gestation (Reveau *et al*., 1998), during which the fetus would acquire 75% of its birth weight (Nadon, 2017). Ensuring the mother receives sufficient inputs is crucial for maintaining her physiological functions and providing the necessary energy and nutrients for fetal growth (Aissaoui *et al*., 2019). The influence of maternal factor in the first 30 days of life of goat kids is very important but after 30 days this factor tend to decrease and in general the accumulation of body mass is provided by the consume of goat milk and also by additional feed. (Călin *et al.*, 2015).

The lowest average body weight at 30 days of age was found in Saanen goat kids (7.66 kg) and the highest value was found in Alpine (7.88kg), a small differences between breeds which is not statistically significant (F=0.318, p=0.575).

The average body weight of Alpine and Saanen kids at 60 days was 13.01 kg and 12.43 kg respectively. Saanen breed displayed a lower body weight but again this difference is not statistically significant (F=0.709, p=0.404).

For Alpine breed were observed higher values of body weight in the three different periods of life, but this difference is not significant. So we can say that in this case the breed does not affect body weight, p>0.05 at three weighing periods. (t-test and ANOVA). F=0.707, p=0.405. The F tests the effect of Breed. Both Alpine and Saanen are imported milk breeds similar to each other and the feeding level practiced in ATTC of Korca is the same for both breeds. According to R. Rojo-Rubio *et al,* (2016) study, the pre-weaning weight, weaning weight (WW), and daily weight gain (DWG) of kids from Alpine (AG), Saanen (SG), and Anglo-Nubian (ANG) breeds were unaffected by the type of kidding and breed, and were higher in males than in females. There may be individual variations, but the breed does not significantly affect the body weight at the specified ages when compared across these breeds.

Regarding the intensity of the growth, the same increasing trend is observed for Alpine and Saanen breeds, with similar values, having an average daily increase (ADG) of 0.157±0.041 kg from 0 to 60 days for Alpine kids and for Saanen breed 0.147±0.032 kg. The average weight gain for Alpine kids from 0 to 60 days was 9.42±2.463 kg compared with Saanen kids which reached a lower weight gain 8.93±1.949 kg. Alpine kids achieved an average daiy gan and a total weight gain 6.8% and 5.5% higher compare to Saanen kids, proving a better capacity to convert milk to body mass (ICDCOC Palas, 2010).

The average body weight of kids at birth, variance, the minimum and maximum values and the significance of the statistical differences for Alpine and Sannen breed are shown in the table below (Table 2).

Many factors influence the growth of the animals and the most important are breed, sex, litter size, age of mother, nutrition, and breeding management (Janos *et al*., 2018). Also growth technology, maintenance level, and microclimatic factors influence the growth and development from birth to weaning.

Table 2. The average birth weight (kg), variance, the minimum and maximum values, confidence interval and the significance of the statistical differences

recorded for Alpine and Saanen goats according to the type of birth and sex of goat kids (kg)

| | Alpine | | | Saanen | | |
|---|---|---|---|---|---|---|
| n | 8 | 16 | 3 | 8 | 12 | 3 |
| type of birth | single | double | triple | single | double | triple |
| Min | 3.00 | 2.00 | 3.00 | 3.00 | 3.00 | 2.50 |
| Max | 4.50 | 4.50 | 3.50 | 4.50 | 3.50 | 3.00 |
| Mean | 3.937 | 3.500 | 3.166 | 4.000 | 3.333 | 2.833 |
| SD | 0.495 | 0.632 | 0.288 | 0.597 | 0.246 | 0.288 |
| CI 95% | 3.523-4.351 | 3.163-3.837 | 2.449-3.883 | 3.50-4.499 | 3.176-3.489 | 2.116-3.55 |
| V | 0.246 | 0.400 | 0.083 | 0.357 | 0.061 | 0.083 |
| p value | | * | ** | | ** | *** |

| | Alpine | | Saanen | |
|---|---|---|---|---|
| n | 10 | 17 | 15 | 8 |
| sex | M | F | M | F |
| Min | 3.50 | 2.00 | 2.50 | 3.00 |
| Max | 4.50 | 4.50 | 4.50 | 4.50 |
| Mean | 3.850 | 3.441 | 3.533 | 3.437 |
| SD | 0.411 | 0.658 | 0.611 | 0.495 |
| CI 95% | 3.555-4.144 | 3.102-3.779 | 3.194-3.871 | 3.023-3.851 |
| V | 0.169 | 0.434 | 0.374 | 0.246 |
| p value | * | | | ns |

M-male; F-female; V-variance; CI-confidence interval; MEAN-average; SD-standard deviation * p<0.05, ** p<0.01, *** p<0.001

Birth weight was influenced by the type of birth and significant differences were observed in the Alpine and Saanen breeds. Single born Alpine kids had higher birth weight (3.937 kg) compared with twins (3.50 kg) and triple born kids (3.166kg). Higher differences were observed between single and triples birth (p<0.01). Also single birth of Saanen kids had higher birth weight (4 kg) followed by twin birth (3.33 kg) and the lower value of birth weight was recorded in triple birth (2.83 kg). The difference is statistically significant (p<0.001). R=0.325, so 32.5% of variation in birth weight is explained by the factor of litter size. The kids from single birth have a better growth rate (Pascal *et al*, 2011). Single kids tended to have greater weights than twins and triples during all the experimentation, (Figure 3). As reported in Doizé *et al*. (2013), and Meza-Herrera *et al*. (2014), cited by Nadon (2017) the type of birth had a significant effect on birth weight; a similar result was reported in Burundi kids (Djıbrıllou, 1986).

It was appeared that competition between fetuses for nutrients and uterine space increased with fetus' number during gestation, reducing their weights (Lawrence *et al.*, 2012). In a study conducted by Amoah *et al.* (1996) on several goat breeds including Alpine and Saanen, it was found that each increase of one fetus per litter was associated with a weight decrease of 0.45 kg per kid. Similar were our values where Alpine single birth kids were 0.437 heavier than twin birth kids. For Saanen breed the difference was higher (0.666 kg).

Differences were observed also between male and female kids. (Figure 3). Females of Alpine breed tended to be lighter compared to males throughout the study.

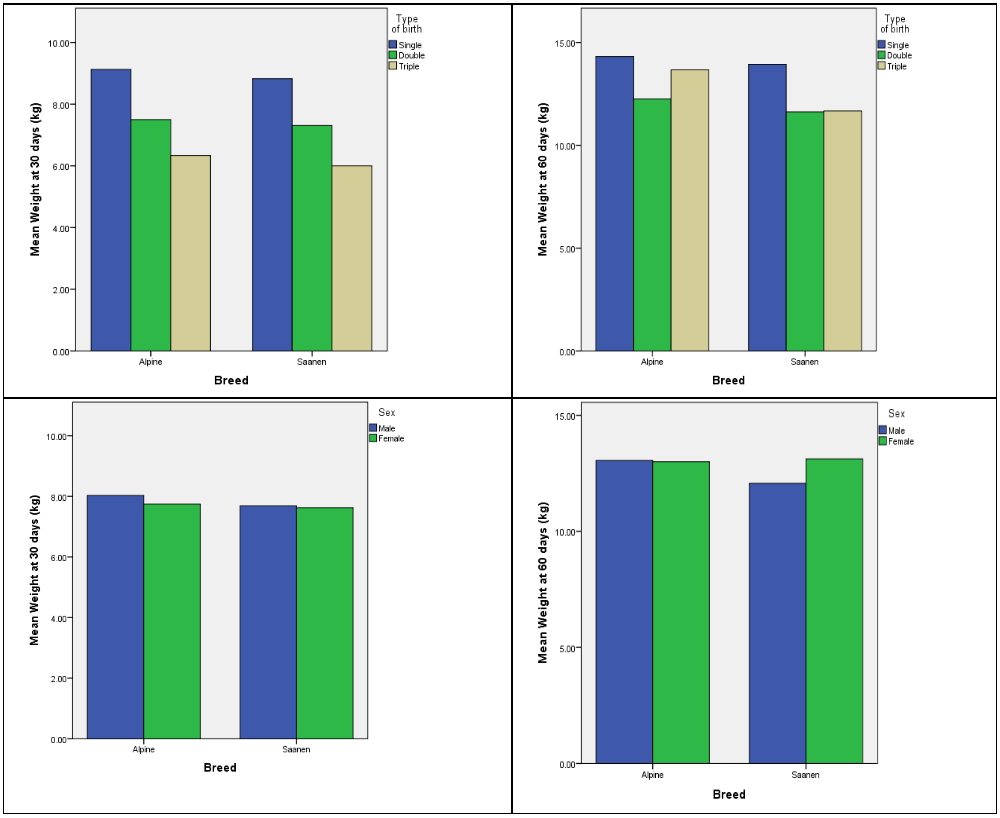


Figure 3. Variation of weight at 30 and 60 days by litter size and sex in Alpine and Saanen goat kids

The average birth weight for Alpine male goat kids was 3.85 kg and for female 3.44 kg the significant difference ($p<0.05$). Saanen male kids had slightly higher value of birth weights compared to females, 3.53 kg and 3.43 kg recpectively, but the difference was not significant (p>0.05).

In the study conducted by Hagan *et al.* (2012) was found that males were heavier at birth than females with respective weights of 1.25 and 1.15 kg. The same findings were made by Meza-Herrera *et al.* (2014), in Mexico, where was found a significant difference in mean birth weight, by sex (3 kg for males, and 2.77 kg for females).

The difference in growth between the sexes lay in conformation and metabolism; according to Benevent *et al.* (1971), each sex evolved under the control of its own endocrine balance, which favored a more or less important organ development.

The absolute growth rate (A) from 0-30 days for Alpine single kids were 0.172 kg. Twin kids had an absolute growth of 0.133kg and triple 0.105kg (Table 3).

Table 3. Variation of growth indices calculated according to type of birth, kids goat sex for Alpine and Saanen goat to birth at 30 days

| | | Trait | n | A (kg) X±sX | R (%) X±sX | I (%) X±sX | F (%) X±sX |
|---|---|---|---|---|---|---|---|
| 0-30 days | | | | | | | |
| Alpine | birth type | S | 8 | 0.172±0.022 | 132.78±17.42 | 79.50±6.60 | 56.81±3.45 |
| | | D | 16 | 0.133±0.013 | 119.59±35.58 | 73.61±11.80 | 53.57±5.94 |
| | | T | 3 | 0.105±0.009 | 100±0.00 | 66.66±0.00 | 50.00±0.00 |
| | Level of significance | | | ** | * | ns | ns |
| | Sex | M | 10 | 0.139±0.023 | 108.56±12.74 | 70.16±5.33 | 51.89±2.92 |
| | | F | 17 | 0.143±0.029 | 128.83±34.93 | 77.18±11.79 | 55.45±5.97 |
| | Level of significance | | | ns | * | ns | ns |
| Saanen | birth type | S | 8 | 0.160±0.018 | 123.60±27.44 | 75.66±9.82 | 54.73±5.05 |
| | | D | 12 | 0.132±0.023 | 120.71±27.98 | 74.40±11.02 | 54.00±5.95 |
| | | T | 3 | 0.106±0.008 | 113.33±23.09 | 71.89±9.05 | 52.77±4.81 |
| | Level of significance | | | * | * | ns | ns |
| | Sex | M | 15 | 0.138±0.032 | 119.12±29.42 | 73.68±11.32 | 53.61±6.04 |
| | | F | 8 | 0.139±0.012 | 123.80±20.52 | 76.06±7.40 | 55.01±3.80 |
| | Level of significance | | | ns | ns | ns | ns |

*p<0.05, **p<0.01, ns- p>0.05. X - average; sx-standard deviation; V-variance; S-single; D-twin; T-triple; M-male; F-female; A-absolute growth; R-relative growth ; I-growth intensity; F-growth factor

The differences between type of birth were significant (p<0.01). The same situation is for the Saanen breed also, where a higher value of absolute growth was recorded in the single births.

Table 4. Variation of growth indices calculated according to type of birth, kids goat sex for Alpine and Saanen goat from birth to 60 days

| | | Trait | n | A (kg) X±sX | R (%) X±sX | I (%) X±sX | F (%) X±sX |
|---|---|---|---|---|---|---|---|
| 0-60 days | | | | | | | |
| Alpine | birth type | S | 8 | 0.173±0.052 | 266.54±87.52 | 111.82±14.93 | 71.47±6.02 |
| | | D | 16 | 0.145±0.035 | 253.55±50.37 | 110.39±12.00 | 70.94±5.13 |
| | | T | 3 | 0.175±0.014 | 334.92±54.98 | 124.65±8.23 | 76.73±3.17 |
| | Level of segnificance | | | * | * | * | * |
| | sex | M | 10 | 0.153±0.034 | 239.26±51.02 | 107.78±11.04 | 69.88±4.72 |
| | | F | 17 | 0.159±0.045 | 282.42±75.43 | 115.11±13.55 | 72.83±5.58 |
| | Level of segnificance | | | ns | * | * | ns |
| Saanen | birth type | S | 8 | 0.166±0.022 | 253.81±50.37 | 110.82±10.70 | 71.17±4.56 |
| | | D | 12 | 0.138±0.038 | 251.58±82.42 | 109.13±13.87 | 70.37±5.62 |
| | | T | 3 | 0.147±0.012 | 315.55±58.24 | 121.77±8.51 | 75.63±3.26 |
| | Level of segnificance | | | * | * | ns | ns |
| | sex | M | 15 | 0.142±0.024 | 247.47±57.36 | 109.29±11.14 | 70.51±4.65 |
| | | F | 8 | 0.161±0.012 | 285.51±89.48 | 115.26±14.78 | 72.87±5.97 |
| | Level of segnificance | | | * | ** | ns | ns |

* p<0.05, ** p<0.01, ns- p>0.05. X-average; sx-standard deviation; V-variance; S-single; D-twin; T-triple; M-male; F-female; A-absolute growth; R-relative growth; I-growth intensity; F-growth factor

Table 5. Pearson's correlation coefficients between growth parameters and weight at different ages for Alpine and Saanen goat kids

| Variables | W0 | W30 | W60 | A | R | I | F | ADG |
|---|---|---|---|---|---|---|---|---|
| W0 | **1** | | | | | | | |
| W30 | **0.759**** | **1** | | | | | | |
| W60 | **0.451**** | **.480**** | **1** | | | | | |
| A | 0.366* | **0.884**** | 0.362** | **1** | | | | |
| R | -0.509* | 0.153 | -0.118 | **0.586**** | **1** | | | |
| I | **-0.478**** | 0.201 | -0.074 | **0.631**** | **0.992**** | **1** | | |
| F | **-0.463**** | 0.219 | -0.057 | **0.645**** | **0.983**** | **0.999**** | **1** | |
| ADG | 0.231 | 0.326* | **0.973**** | 0.299* | 0.004 | 0.043 | 0.059 | **1** |

The same parameters mentioned above were analyzed for the period from birth to 60 days (Table 4). No significant differences were observed in growth parameters between Alpine and Saanen goat kids. The difference between males and females was not significant for all the growth parameters. Sometimes individual factors influenced more weight and performance variations than sex, or even litter size (Leimbacher and Tatareau, 1991).

Mean values of growth parameters were analyzed and reported in Table 5. Pearson correlation test at two levels of significance (0.05 and 0.01) showed significant correlations, mostly positive, and linking growth parameters (r>0.5). Significant correlations were noted between weights at 30 and 60 days with ADG. Also, significant and strong positive correlations were observed between absolute growth rate and weight at different ages.

## CONCLUSIONS

The type of birth significantly influenced birth weight, with statistical support at p<0.01. Single kids tended to have greater weights than twins and triples. Sex showed a significant influence on birth weight for Alpine goat kids. Females tended to be lighter compared to males throughout the study. Average daily gains from birth to 60 days of kids were 0.157 kg and 0.148 kg for Alpine and Saanen, respectively. Both Alpine and Saanen goat kids realized a satisfactory growth performance.

The differences and variationsin the growth pattern of the Alpine and Saanen kids would be very helpful for better understanding of the production characteristics of these breeds. The results of the study canserve to goats breeder, in choosing the breed that fits better to their farming system and improve the production level for both milk and meat. These breeders can be put in the genetic breeding program. Growth data can be used for selective breeding programsconducted by ATTC of Korca to improve growth rates and overall productivity.

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# NUTRITIONAL STATUS OF YOUNG SCHOOL-AGED CHILDREN IN PODGORICA, MONTENEGRO

## SUMMARY

Excess body weight and obesity, alongside undernutrition, are increasingly prevalent in school populations. Monitoring these disorders is crucial for understanding the societal situation and for timely interventions to prevent their occurrence. The aim of this research was to determine the morphological characteristics and body composition of children in elementary school and to highlight the importance of addressing the causes of any deviations from physiological limits since the nutritional status of children at an early school age is the best indicator of their proper growth and development. Through this objective, the importance of implementing strategies and plans for proper nutrition of children at all ages was also emphasized. The study was conducted at the Elementary School in Podgorica and included 823 students aged 6 to 15 years, 375 girls and 448 boys. During the study, the following parameters were measured: height, weight, upper arm circumference, and skinfold thickness at the triceps. Based on the measured parameters, body mass index (BMI), muscle mass, and fat mass were determined. All collected data were exported to the SPSS program. Boys grew by 55.82 cm and girls by 43.18 cm from ages 6 to 15. There was no significant height advantage until age 13, when boys significantly surpassed girls. At ages 13, 14, and 15, boys averaged 5 to 9 kg more than girls. Obesity among girls was present across all age groups, with the lowest prevalence at age 7 and the highest at age 9, at 25.49%. The highest percentage of obesity in boys was recorded at age 10, at 34.88%. A negative correlation was found between BMI and muscle mass (-0.197), as well as between BMI and fat mass (-0.524). Although the majority of children have a normal nutritional status, it is concerning that the highest number of obese children is among those aged 9 and 10. According to WHO program recommendations, measures and strategies should be implemented to support healthy nutrition and promote health.

**Keywords**: nutritional status, school population, growth and development, obesity

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## INTRODUCTION

The modern lifestyle, along with technological development, has influenced all areas of society. This impact has also reached younger age groups, who adapt easily to innovations, particularly noticeable in urban environments (Scepanovic *et al.*, 2019). Numerous studies have established that this has led to an increase in eating disorders among children. Overweight, obesity, and body fat are prevalent, while undernutrition also exists. The location of fat tissue in the human body determines its metabolic profile. 50-70% of the total body fat mass is stored in subcutaneous fat, while the remainder is the so-called visceral fat, which is located beneath the abdominal wall. Increased energy intake and reduced energy expenditure lead to greater fat deposition and an increase in fat tissue (obesity) (Pavlica, Rakic, 2019). Monitoring these disorders is crucial for understanding the societal situation and for timely interventions to prevent their occurrence, through campaigns promoting healthy lifestyles and proper nutrition, with reduced participation of industrial products rich in trans fats, sugars, and additives (WHO, 2005). In this regard, the European WHO initiative for monitoring childhood obesity (COSI) was established in response to the need for standardized data on the prevalence of overweight and obesity among school-aged children (WHO, 2022). Access to such information is key for developing effective policies and strategies to combat childhood obesity. Meeting nutritional needs and ensuring food safety are important factors in preventing a range of diseases and disorders. In light of this, the Ministry of Health of Montenegro developed the Action Plan for Nutrition and Food Safety in Montenegro (2010-2014) to improve public health protection. The Action Plan for Nutrition and Food Safety in Montenegro serves as a framework document that provides basic guidelines and a coordinated response to the increasing public health challenges related to food and nutrition (Action Plan for Nutrition and Food Safety in Montenegro, 2011). In recent decades, the childhood obesity epidemic has been on the rise (Kimm and Obarzanek, 2002; Ogden *et al*., 2002, 2014). Its prevalence has reached epidemic levels (Branca *et al.*, 2007; Swinburn *et al*., 2011). Based on previous research, the global prevalence of obese children has doubled, while the number of obese adolescents has quadrupled (Ogden *et al.*, 2014; Health, United States, 2011). Numerous data indicate the negative impact of obesity on the proper growth and development of children. It has also been found that these negative impacts are more pronounced in girls than in boys (Drid *et al.*, 2013; Kostic *et al.*, 2009). Additionally, obese children and adolescents are more likely to be obese in adulthood. This is supported by a study conducted by Wang and Lobstein (2006), which demonstrated that children with excess body weight by the age of eight are at greater risk of obesity in adulthood. Data on the health and nutrition of the population in Montenegro are quite limited.

The Multiple Indicator Cluster Survey in Montenegro from 2018 (MICS, 2019) provided representative data related to nutrition; however, only anthropometric markers for preschool-aged children were analyzed, while assessments for other population groups regarding nutrition were not conducted. The aim of this study was to determine the morphological characteristics and body composition of young school-aged children in the urban center of Podgorica and to highlight the importance of investigating the causes of any deviations from physiological standards for that age group. This is crucial because the nutritional status of children in early school age is the best indicator of their proper growth and development. Through this goal, the research also highlighted the importance of implementing strategies and plans for proper nutrition for children at all ages.

## MATERIAL AND METHODS

The study was conducted during the year 2022. at the "21.maj" Elementary School in the urban center of Podgorica and included 823 students aged 6 to 15 years. There were 375 girls (45.56%) and 448 boys (54.43%). During the study, the following parameters were measured and analyzed: height, weight, upper arm circumference, and skinfold thickness at the triceps. Body Mass Index (BMI) was obtained as the ratio of body mass to the square of body height, using the formula (kg) / ($m^2$). Nutritional status was then determined using percentile values. Additionally, muscle and fat mass were calculated from the measured values of upper arm circumference and skinfold thickness at the triceps (Frisancho, 1990). Standardized anthropological instruments were used for anthropometric assessment: anthropometer, scale, and caliper. Statistical analysis of the measured values was performed using standard statistical methods in the SPSS program, including descriptive statistics, correlation analysis, and hypothesis testing using ANOVA. This research was conducted in accordance with the Declaration of Helsinki, with the consent of all parents and school principals. Each parent-participant voluntarily provided written information and consent prior to participating in this study.

## RESULTS AND DISCUSSION

Table 1 presents the descriptive statistics for the parameters of height and weight in the overall sample according to gender and age from 6 to 15 years.

Based on the data, the average height increase observed shows that boys grow by 55.82 cm from ages 6 to 15, while girls grow by 43.18 cm. Between the ages of 7 and 13, both girls and boys have approximately the same average weight. However, at ages 13, 14, and 15, boys weigh on average 5 to 9 kg more than girls.

Table 2 presents the analysis of the statistical significance of height values among students of both genders in relation to age. Variance analysis revealed a statistically significant difference in height between boys and girls at ages 8, 14, and 15, which is expected given the known growth rate during this developmental period.

Table 1. Height and weight of students by gender and age

| age | gender | N | Mean height | Std. deviation | Min | Max | Mean weight | Std. deviation | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | boys | 10 | **121.0600** | 5.56581 | 111.00 | 130.10 | 26.5286 | 6.50172 | 18.10 | 37.60 |
| | girls | 7 | **122.7286** | 5.57576 | 114.60 | 128.90 | 26.0500 | 6.45002 | 16.80 | 38.40 |
| 7 | boys | 56 | **127.7982** | 6.2848 | 115.50 | 143.30 | 20.0911 | 7.10140 | 18.60 | 56.40 |
| | girls | 45 | **126.5644** | 6.3529 | 116.30 | 142.60 | 26.1178 | 4.23654 | 19.30 | 37.40 |
| 8 | boys | 38 | **136.2947** | 7.1379 | 123.70 | 150.10 | 33.3921 | 7.77290 | 16.30 | 53.00 |
| | girls | 34 | **131.7941** | 7.6330 | 118.40 | 155.80 | 29.8471 | 5.60672 | 17.90 | 46.60 |
| 9 | boys | 56 | **139.6482** | 6.17744 | 124.00 | 153.60 | 35.7054 | 7.33288 | 23.20 | 53.20 |
| | girls | 59 | **139.5549** | 5.57707 | 125.80 | 152.20 | 35.6471 | 7.07178 | 23.50 | 49.60 |
| 10 | boys | 43 | **144.9256** | 7.72621 | 128.70 | 161.30 | 40.5837 | 9.08083 | 25.70 | 65.60 |
| | girls | 32 | **144.0875** | 6.31689 | 133.50 | 159.00 | 39.4563 | 7.56426 | 27.10 | 57.60 |
| 11 | boys | 59 | **151.0627** | 7.37979 | 131.50 | 168.30 | 45.2593 | 10.52668 | 28.00 | 79.00 |
| | girls | 46 | **150.3457** | 9.07903 | 129.40 | 167.00 | 42.1022 | 8.98791 | 26.80 | 60.90 |
| 12 | boys | 47 | **156.9340** | 8.74129 | 140.50 | 193.70 | 49.3106 | 10.16238 | 29.40 | 79.10 |
| | girls | 49 | **159.1224** | 7.12210 | 143.00 | 174.40 | 52.2143 | 12.21550 | 31.30 | 90.40 |
| 13 | boys | 48 | **164.1333** | 11.7395 | 140.50 | 191.40 | 59.6292 | 15.12099 | 32.40 | 100.80 |
| | girls | 44 | **161.1592** | 6.52103 | 146.60 | 174.50 | 54.4386 | 9.95600 | 32.70 | 80.00 |
| 14 | boys | 61 | **172.2262** | 8.99866 | 157.50 | 191.50 | 65.9967 | 14.57817 | 39.00 | 98.20 |
| | girls | 38 | **163.4921** | 7.06340 | 152.00 | 182.70 | 56.0816 | 8.65169 | 38.00 | 74.60 |
| 15 | boys | 33 | **176.8818** | 8.22255 | 162.10 | 198.00 | 68.1818 | 12.39633 | 46.40 | 94.00 |
| | girls | 26 | **165.9000** | 5.33547 | 156.00 | 173.90 | 59.9885 | 10.36947 | 40.30 | 80.40 |

Table 2. Analysis of variance – body height among students of both genders in relation to age.

| age | height | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 6 | Between Groups | 11.464 | 1 | 11.464 | .370 | .552 |
| | Within Groups | 465.338 | 15 | 31.023 | | |
| | Total | 476.802 | 16 | | | |
| 7 | Between Groups | 37.979 | 1 | 37.979 | .952 | .332 |
| | Within Groups | 3948.353 | 99 | 39.882 | | |
| | Total | 3986.332 | 100 | | | |
| 8 | Between Groups | 363.475 | 1 | 363.475 | 6.682 | **.012*** |
| | Within Groups | 3807.858 | 70 | 54.398 | | |
| | Total | 4171.333 | 71 | | | |
| 9 | Between Groups | .232 | 1 | .232 | .007 | .935 |
| | Within Groups | 3654.026 | 105 | 34.800 | | |
| | Total | 3654.259 | 106 | | | |
| 10 | Between Groups | 12.886 | 1 | 12.886 | .251 | .618 |
| | Within Groups | 3744.157 | 73 | 51.290 | | |
| | Total | 3757.043 | 74 | | | |
| 11 | Between Groups | 13.290 | 1 | 13.290 | .199 | .656 |
| | Within Groups | 6868.052 | 103 | 66.680 | | |
| | Total | 6881.342 | 104 | | | |
| 12 | Between Groups | 114.889 | 1 | 114.889 | 1.815 | .181 |
| | Within Groups | 5949.631 | 94 | 63.294 | | |
| | Total | 6064.520 | 95 | | | |
| 13 | Between Groups | 203.076 | 1 | 203.076 | 2.200 | .141 |
| | Within Groups | 8305.973 | 90 | 92.289 | | |
| | Total | 8509.049 | 91 | | | |
| 14 | Between Groups | 1786.146 | 1 | 1786.146 | 36.209 | **.000*** |
| | Within Groups | 4784.866 | 97 | 49.329 | | |
| | Total | 6571.012 | 98 | | | |
| 15 | Between Groups | 1753.815 | 1 | 1753.815 | 34.769 | **.000*** |
| | Within Groups | 2875.209 | 57 | 50.442 | | |
| | Total | 4629.024 | 58 | | | |

In Table 3, the analysis of the statistical significance of body weight values among students of both genders in relation to age is presented. The results show that there is a statistically significant difference in body weight values at ages 7, 8, 13, 14, and 15.

Table 3. Analysis of variance – body weight among students of both genders in relation to age.

| age | weight | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 6 | Between Groups | .943 | 1 | .943 | .023 | .883 |
| | Within Groups | 628.059 | 15 | 41.871 | | |
| | Total | 629.002 | 16 | | | |
| 7 | Between Groups | 220.574 | 1 | 220.574 | 6.128 | .015* |
| | Within Groups | 3563.371 | 99 | 35.994 | | |
| | Total | 3783.946 | 100 | | | |
| 8 | Between Groups | 225.514 | 1 | 225.514 | 4.823 | .031* |
| | Within Groups | 3272.832 | 70 | 46.755 | | |
| | Total | 3498.347 | 71 | | | |
| 9 | Between Groups | .091 | 1 | .091 | .002 | .967 |
| | Within Groups | 5457.915 | 105 | 51.980 | | |
| | Total | 5458.006 | 106 | | | |
| 10 | Between Groups | 23.322 | 1 | 23.322 | .325 | .570 |
| | Within Groups | 5237.137 | 73 | 71.742 | | |
| | Total | 5260.459 | 74 | | | |
| 11 | Between Groups | 257.638 | 1 | 257.638 | 2.637 | .107 |
| | Within Groups | 10062.252 | 103 | 97.692 | | |
| | Total | 10319.890 | 104 | | | |
| 12 | Between Groups | 202.260 | 1 | 202.260 | 1.596 | .210 |
| | Within Groups | 11913.085 | 94 | 126.735 | | |
| | Total | 12115.345 | 95 | | | |
| 13 | Between Groups | 618.486 | 1 | 618.486 | 3.709 | .037* |
| | Within Groups | 15008.523 | 90 | 166.761 | | |
| | Total | 15627.009 | 91 | | | |
| 14 | Between Groups | 2301.845 | 1 | 2301.845 | 14.386 | .000* |
| | Within Groups | 15520.896 | 97 | 160.009 | | |
| | Total | 17822.742 | 98 | | | |
| 15 | Between Groups | 976.245 | 1 | 976.245 | 7.316 | .009* |
| | Within Groups | 7605.556 | 57 | 133.431 | | |
| | Total | 8581.801 | 58 | | | |

*p<0,05

Table 4. Nutritional status of girls aged 6 to 15 years

| age | N Girls | malnutrition N (%) | normal BMI N (%) | preobesity N (%) | obesity N (%) |
|---|---|---|---|---|---|
| **6** | 10 | 0 (0%) | 7 (70%) | 2 (20%) | 1 (10%) |
| **7** | 45 | 1 (2,22%) | 34 (75,55%) | 8 (17,77%) | 2 (4,44%) |
| **8** | 34 | 1 (2,94%) | 21 (61,76%) | 10 (29,41%) | 2 (5,88%) |
| **9** | 51 | 2 (3,92%) | 29 (56,86%) | 7 (13,72%) | 13 (25,49%) |
| **10** | 32 | 1 (3,12%) | 17 (53,12%) | 10 (31,25%) | 4 (12,5%) |
| **11** | 46 | 2 (4,34%) | 31 (67,39%) | 9 (19,56%) | 4 (8,69%) |
| **12** | 49 | 2 (4,08%) | 32 (65,30%) | 6 (12,24%) | 9 (18,36%) |
| **13** | 44 | 1 (2,27%) | 31 (70,45%) | 6 (13,63%) | 9 (20,45%) |
| **14** | 38 | 2 (5,26%) | 29 (76,31%) | 5 (13,15%) | 2 (5,26%) |
| **15** | 26 | 1 (3,84%) | 20 (76,92%) | 3 (11,53%) | 2 (7,69%) |
| **Ukupno** | **375** | **13 (3,46%)** | **248 (66,13%)** | **66 (17,60%)** | **48 (12,80%)** |

BMI, calculated as the ratio of body weight to height, for the examined age group is expressed in percentile values, based on which the classification of this index was made in relation to gender and age. The results are presented in the tables below.

For all age groups, the percentage of underweight girls is low, with only 5.26% of underweight girls at age 14. Regarding normal body weight, the lowest representation was at ages 8 and 9, with 53%, while in other age groups it ranged from 60% to 77%. The highest prevalence of excess body weight was observed in the ages of 8 and 10. Obesity was present in all age groups, with the lowest prevalence at age 7 and the highest at age 9, at 25.49%.

In the same way, Table 5 presents the results of the nutritional status for boys by age groups.

Table 5. Nutritional status of boys aged 6 to 15 years

| age | N boys | malnutrition N (%) | normal BMI N (%) | preobesity N (%) | obesity N (%) |
|---|---|---|---|---|---|
| **6** | 7 | 0 (0%) | 5 (71,41%) | 2 (28,57%) | 0 (0%) |
| **7** | 56 | 0 (0%) | 31 (55,35%) | 9 (16,07%) | 16 (28,57%) |
| **8** | 38 | 1 (2,63%) | 22 (57,89%) | 10 (26,31%) | 5 (13,15%) |
| **9** | 56 | 0 (0%) | 31 (55,35%) | 11 (19,64%) | 14 (24,99%) |
| **10** | 43 | 0 (0%) | 21 (48,83%) | 7 (16,27%) | 15 (34,88%) |
| **11** | 59 | 1 (1,69%) | 18 (30,50%) | 26 (44,06%) | 14 (23,72%) |
| **12** | 47 | 3 (6,38%) | 23 (48,93%) | 12 (25,53%) | 9 (19,14%) |
| **13** | 48 | 2 (4,16%) | 23 (47,91%) | 9 (18,74%) | 14 (29,16%) |
| **14** | 61 | 2 (3,27%) | 32 (52,45%) | 12 (19,63%) | 15 (24,59%) |
| **15** | 33 | 4 (12,12%) | 19 (57,57%) | 7 (21,21%) | 3 (9,09%) |
| **Ukupno** | **448** | **13 (2,90%)** | **225 (50,22%)** | **105 (23,43%)** | **105 (23,43)** |

At ages 6, 7, 9, and 10, undernutrition was not observed, while the highest percentage of underweight individuals was noted at age 15, with over 12%. Also, in all age groups, the largest number of boys had normal body weight, with the lowest values recorded at age 11, at 30.50%. The prevalence of excess weight was lowest at ages 7 and 10, at 16%, and highest at age 11, at 44.06%. Regarding obesity, it was not observed at age 6, while the highest percentage of obesity among boys was recorded at age 10, at 34.88%.

Based on the measured values of upper arm circumference and skinfold thickness at the triceps, the values for muscle and fat components were calculated, which are presented in Table 6.

It is observed that from ages six to eight, there is a trend of increasing muscle mass in both genders. From age eight onward, the values of muscle mass begin to decline. Additionally, up to age ten, girls, on average, have higher values than boys. Based on the data in the table, it is clear that the lowest value of fat mass is for the age of 14, while the highest is for the age of 6. From these values, we can conclude that fat mass decreases with age.

Analyzing the correlations between the variables BMI, muscle mass, and fat mass, the results showed a negative correlation between BMI and muscle mass (-0.197), as well as between BMI and fat mass (-0.524). The relationship between

BMI and fat mass is stronger, but negative; with a 1 percentage point increase in BMI, there is a decrease in fat mass of 0.524 percentage points. On the other hand, as expected, there is a negative correlation between muscle mass and fat mass; with a 1 percentage point increase in muscle mass, there is a decrease in fat mass of 0.195 percentage points. Detailed data is provided in Table 7 below.

Table 6. Muscle and fat component

| age | gender | N | Mean muscle mass | Std. deviation | Min | Max | Mean fatty mass | Std. deviation | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|
| **6** | boys | 7 | 13.4371 | 4.12136 | 9.42 | 21.23 | 29.4286 | 8.62104 | 11.00 | 35.60 |
| | girls | 10 | 17.8810 | 6.87043 | 6.43 | 28.62 | 27.0500 | 13.35367 | 11.40 | 47.80 |
| **7** | boys | 56 | 23.5916 | 11.60807 | 5.97 | 47.22 | 25.3536 | 10.19920 | 5.40 | 57.60 |
| | girls | 45 | 25.1344 | 12.37251 | 9.49 | 52.25 | 25.8400 | 11.27111 | 1.40 | 51.70 |
| **8** | boys | 38 | 26.4150 | 13.05942 | 8.32 | 59.24 | 22.4342 | 8.31564 | 8.80 | 43.80 |
| | girls | 34 | 27.7456 | 15.50776 | 7.91 | 71.80 | 22.8618 | 7.88278 | 5.40 | 39.50 |
| **9** | boys | 56 | 22.2654 | 10.61007 | 8.35 | 50.90 | 22.1839 | 7.46338 | 8.00 | 41.40 |
| | girls | 51 | 21.1449 | 9.93186 | 9.46 | 50.24 | 23.6784 | 8.77540 | 8.60 | 47.20 |
| **10** | boys | 43 | 23.1870 | 10.92435 | 8.11 | 61.16 | 19.2419 | 5.98089 | 5.80 | 30.90 |
| | girls | 32 | 24.4219 | 11.21975 | 8.05 | 52.30 | 17.8938 | 5.80089 | 7.00 | 32.80 |
| **11** | boys | 59 | 26.3100 | 12.91470 | 7.95 | 59.91 | 16.3763 | 5.03393 | 2.80 | 27.00 |
| | girls | 46 | 24.7157 | 10.38250 | 8.83 | 51.46 | 17.6261 | 6.65006 | 3.70 | 37.90 |
| **12** | boys | 47 | 23.1749 | 11.55709 | 7.58 | 63.00 | 17.2660 | 7.15913 | 5.80 | 37.10 |
| | girls | 49 | 21.6792 | 9.72877 | 8.05 | 45.16 | 16.500 | 5.27964 | 2.00 | 28.00 |
| **13** | boys | 48 | 20.5523 | 10.12719 | 8.28 | 61.77 | 14.4583 | 5.66564 | 7.80 | 32.70 |
| | girls | 44 | 20.3843 | 9.80145 | 6.96 | 45.14 | 16.8045 | 6.94878 | 3.30 | 39.10 |
| **14** | boys | 61 | 23.7392 | 12.24567 | 8.83 | 58.56 | 13.5262 | 5.17001 | 2.00 | 25.20 |
| | girls | 38 | 20.5579 | 9.76805 | 7.10 | 43.68 | 12.9658 | 4.90726 | 4.10 | 27.60 |
| **15** | boys | 33 | 17.4933 | 7.33636 | 7.78 | 36.54 | 14.8667 | 3.69803 | 9.30 | 23.00 |
| | girls | 26 | 20.5554 | 8.13552 | 9.23 | 39.31 | 13.6000 | 3.80421 | 4.80 | 21.90 |

Table 7. The degree of correlation between BMI, muscle mass, and fat mass

**Correlations**

| | | BMI | muscle mass | fatty mass |
|---|---|---|---|---|
| BMI | Pearson Correlation | 1 | -.197** | -.524** |
| | Sig. (2-tailed) | | .000 | .000 |
| | N | 823 | 823 | 823 |
| muscle mass | Pearson Correlation | -.197** | 1 | -.195** |
| | Sig. (2-tailed) | .000 | | .000 |
| | N | 823 | 823 | 823 |
| fatty mass | Pearson Correlation | -.524** | -.195** | 1 |
| | Sig. (2-tailed) | .000 | .000 | |
| | N | 823 | 823 | 823 |

**. Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

In childhood, disorders related to body weight often arise from inadequate nutrition. Food that is low in nutrients but high in energy value is readily available to the population. The consumption of such foods by children, combined with decreased physical activity, increases the risk of overweight and obesity (Medanic, Pucarin-Cvetkovic, 2012). Overweight or obesity are becoming so prevalent that they are approaching epidemic levels, as evidenced by the health statistics of children worldwide. This issue is becoming increasingly pronounced in developed countries, and research conducted so far indicates a growing trend of children with excess body weight and obesity, corroborated by data from the World Health Organization (WHO, 2022). According to the findings of the MICS in 2018, 7.3% of children under five years old were overweight. In the following year, 2019, the Institute for Public Health of Montenegro conducted a survey that included seven-year-olds, which revealed that 18.1% of boys and girls were overweight, while 16.1% of boys and 10.5% of girls were obese (Institute of Public Health of Montenegro, UNICEF, and GroundWork (2022). The program of measures for improving nutritional status in Montenegro, along with the Action Plan for the period 2021-2022, emerged from the need to continue aligning national activities in this area with regional recommendations from the World Health Organization related to the European region (European Action Plan on Food and Nutrition for the period 2015-2020). It also aims to adopt recommendations highlighted in the Final Report on the implementation of the Action Plan (Program of Measures for Improving Nutritional Status in Montenegro for 2020). During the three-year period between two rounds of the COSI study (2016 and 2019), the Institute for Public Health of Montenegro, with support from UNICEF, developed and published Guidelines for the Nutrition of Preschool-Aged Children and Recommendations for Reducing the Intake of Foods High in Saturated Fats, Trans Fats, Sugar, and Salt, with the aim of improving children's nutrition and creating an environment that promotes and supports proper nutrition (WHO, COSI, 2022).

Montenegro joined this research during the fourth round of investigations in 2016. The target group comprised seven-year-olds. Their anthropometric characteristics were measured, and parents provided data on dietary habits, physical activity, and the socio-economic characteristics of the family. In 2019, data revealed that nearly one-fifth of girls and boys had excess body weight (18.1%), while just under one-fifth were obese (16.1%). Girls had nearly the same percentage of excess weight as boys, while almost one in five boys (16.1%) and one in ten girls (10.5%) were obese. According to this research, the average body weight for seven-year-olds was 29.0 kg, the average height was 129.3 cm, and the average body mass index was approximately 16.7 kg/m$^2$. Comparing these results with those of our research, smaller deviations are observed in terms of height, weight, and body mass index. (For the same age group, these results are on average: body weight 27.76 kg, body height 127.24 cm, and the average BMI value 16.9 kg/m$^2$). When discussing the daily consumption of fresh fruit among children aged 6-9 years in Montenegro, it is noticeable that girls consume fruit more frequently than boys, with a difference of 6%, while boys more often consume sugary soft drinks compared to girls (28% versus 24%) (WHO, COSI, 2022). Numerous studies have been conducted in neighboring countries concerning body weight and nutrition among children. For example, the Croatian Institute of Public Health, in collaboration with UNICEF (2024), provided a series of recommendations for the prevention of overweight and obesity in children through a review of research, practices, and documents, after finding that Croatia ranks fifth in Europe in relation to these health issues. For the same reasons, the Public Health Institute of the Federation of Bosnia and Herzegovina (2012) published guidelines for healthy nutrition of preschool and school-age children. Many authors have also addressed this issue using slightly smaller samples than those mentioned. Djukic and Medjedovic (2013), in their work on the nutrition of children aged 9 to 12 years in Serbia, indicated that of a sample of 757 students, 66.3% had a normal body weight, 18.4% were overweight, and 15.3% were obese. They also found that obesity was more common in younger age groups.

Our research showed a lower percentage of children with normal body weight (50.22%) and a higher percentage of children with overweight (23.43%) and obesity (23.43%) compared to the aforementioned study, with obesity also being more pronounced in younger age groups, specifically 24.99% in 9-year-olds and as high as 34.88% in 10-year-olds. To determine the impact of a modern lifestyle on children, particularly nutrition, other anthropometric measures were also examined, as well as their correlation with excess body weight. Many works have investigated the relationship between anthropometric parameters and body mass index to identify groups at risk for overweight and obesity. Tomkinson *et al.* (2018) established that differences between genders in terms of adipose tissue become apparent after the age of twelve, while deviations in our study appeared at ages 6, 10, and 13. At ages 6 and 10, boys had higher fat mass values, while at age 13, girls surpassed boys in this parameter. In all age groups, this difference is around 2%. Herda *et al.* (2016) determined that children with a higher percentage of subcutaneous fat have less muscle mass. Our research also examined the degree of correlation between BMI, muscle mass, and fat mass, and it was found that there is a negative correlation between BMI and muscle mass (-0.197), as well as between BMI and fat mass (-0.524). On the other hand, as expected, there is a negative correlation between muscle mass and fat mass, meaning that for every 1 percentage point increase in muscle mass, fat mass decreases by 0.195 percentage points. In the aforementioned study conducted in 2019, which also included demographic parameters, it was established that in the central part of Montenegro, there was a higher percentage of boys with excess body weight and obesity, which also aligns with the results of our research, indicating an even greater percentage of obesity among boys. Regarding the distribution of children with obesity in relation to the degree of urbanization, it is noticeable that the highest percentage (15%) lives in urban areas compared to suburban (12.3%) and rural areas (9.6%). Unfortunately, numerous studies indicate a significant percentage decrease in the rural population in our country and negative demographic trends that result in a shift towards urban areas. This not only affects the socioeconomic situation but can also negatively impact the overall health of the population. Primarily, this refers to the negative effects of the fast-paced urban lifestyle, the detachment from traditional foods, reduced physical activity, and an increasingly sedentary lifestyle in indoor environments.

The results of obesity studies, particularly among children (Scepanović *et al.,* 2019), as well as the previously mentioned projects carried out in Montenegro across all three regions, also address this issue, including population nutrition. Confirmation of the demographic trends from the north has been provided by research showing the impact of economic and ecological events on migration patterns from Pljevlja and other northern municipalities since the beginning of the 21st century. Emigration of the young and middle-aged population led to the transformation of the agricultural population into a non-agricultural one, which initially had positive effects. However, today, due to the spontaneity of this process, the agricultural population is largely made up of older households, which brings negative consequences in all spheres of life, including those related to food strategies and care (Mijanović *et al.,* 2023).

## CONCLUSIONS

Based on this research and with the help of the criteria and recommendations from the WHO, it is concluded that each new study related to excess body weight and obesity in children yields increasingly poor and concerning results. When it comes to parameters such as height, the typical so-called first crossover of growth curves, where girls surpass boys in height at age 9, is absent; instead, there is a uniformity in height that persists until age 13. Unfortunately, an increased percentage of obese children is already evident at ages 9 and 10. It is essential to undertake urgent measures and strategies for proper nutrition in children within the framework of multidisciplinary activities to avoid more severe health consequences. By focusing on these aspects, we can better understand how children’s nutritional status directly influences their overall well-being. It emphasizes the need for targeted interventions, early identification of potential nutritional issues, and the promotion of healthy eating habits to support their growth. Additionally, it points to the role of schools, families, and communities in ensuring that children receive adequate nutrition, which is essential for their physical and cognitive development.

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# PEER LEARNING AND KNOWLEDGE CO-CREATION IN ORGANIC AQUACULTURE: THE CASE OF FUTUREEUAQUA PROJECT

## SUMMARY

Organic aquaculture represents 4.6% of total aquaculture production. However, the economic results of organic aquaculture in the EU appear to be far from satisfying. Notwithstanding the above, Europe is still heavily dependent on external markets to cover seafood demand. Nowadays, fisheries and aquaculture sectors require paramount need to (i) enhance knowledge sharing, (ii) set up appropriate infrastructures and facilities (iii) increase capacity building. To overcome these needs an “active and participative” training method that allows participants to fully learn news concepts, while improving skills and capabilities, could be adopted. One of the most profitable methods is known as “Peer learning”, a form of collaborative instruction that enhances peer-to-peer interaction and leads to positive learning results. Based on this approach, CIHEAM Bari has created and led an online training course on "Sustainable, resilient and climate friendly Blue Growth of EU Aquaculture and beyond."

The course involved 357 participants coming from 77 countries. About 50 participants achieved the required score to receive the certificate as a result of the dropout phenomenon. With the ultimate goal of converting results and outputs into practical knowledge when putting the creative solution found and developed within the project into practice, the course's outcomes aimed to raise awareness of the industry by establishing a community of practice where everyone shares their technical and social knowledge.

It may be concluded that adopting appropriate technology for eLearning and innovative educational approach may fill the gap of knowledge transfer under disruptive circumstance (i.e. COVID-19 pandemic; etc.).

**Keywords**: Education, eLearning, Organic aquaculture, Communities of practices.

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## INTRODUCTION

Marine environments are vital for ecosystem services that underpin planetary health and societal needs (Peterson and Lubchenco, 1997). They provide 16% of global animal protein, supporting significant economic activities and employment, particularly in developing countries where over 41 million people work in fish production and food security (Tacon and Metian, 2013; Finegold, 2009; Béné *et al.*, 2016). Fisheries and aquaculture play a critical role in addressing nutritional needs, especially in vulnerable communities, and driving economic development (Ferreira, Rice and Rosenberg, 2016).

With global fisheries largely exploited and production plateauing in recent years (Garcia and Newton, 1995), aquaculture has become the fastest-growing food production system, achieving an annual growth rate of approximately 9% since 1985 (Diana, 1993). Overfishing, stock collapse, maritime traffic, and water contamination makes fishery production alone insufficient to meet global food demand (Simcock, 2017). Thus, aquaculture development is seen as a strategic solution to fisheries depletion, leveraging resources already present in aquaculture systems (Longo *et al.*, 2019).

However, as aquaculture progress, it becomes essential for governments to proactively manage its potential ecological and social impacts (Finegold 2009).

In 2015, world aquaculture production reached over 100 million tons, while European aquaculture production reached 2.98 million tons of seafood, with a value over 11 billion USD. Some models predict that the amount of total fish supply will increase until 186 million tons in 2030, with aquaculture entirely responsible for this increase. (Kobayashi *et al.* 2015).

Despite the worldwide increasing production in aquaculture, organic aquaculture represents only 4.7% of the total EU aquaculture production. Moreover, the interest in organic aquaculture has grown in recent years (Lembo and Mente 2019).

So, to answer to these needs, it is urgent to form new skilled professional figure, attract new stakeholders and improve the awareness of the sector in particular: enhancing knowledge sharing and co-creation, (iii) setting up-appropriate infrastructures and facilities and (iii), increase capacity building for experts and practitioners as also underlined by Agenda2030 (SDGs 14) (Colglazier 2015) promoting good governance, best practices in fisheries and inclusive decision-making procedures.

There are different methods to create new expertise through training courses. One of the most profitable method is known as “Peer learning”, a form of collaborative instruction that enhances the peer to peer interaction and leads to positive learning results (Topping 2013). Among the advantages are the growth of students' communication and teamwork abilities, their confidence, and their capacity to direct their own education. Compared to a teacher-led setting, trainers may interact, reflect, and go deeper into ideas when working with their peers since they feel more at ease doing so.

As a result of the "collaborative-constructivist" methodology being used in the particular context of Alumni, or adult participants, favourable conditions were created for the development of a community of practice on topics of shared interest. (Driouech *et al.* 2015).

Follow-up studies are frequently employed by academic and research institutions in the context of alumni networking to review and assess the efficacy of their educational and academic programs.

This research highlights the critical importance of the online training course designed under the framework of the FutureEUAqua project in shaping new professional profiles in organic aquaculture. The course goes beyond conventional training by addressing future challenges such as growing consumer demand for high-quality, nutritious, and responsibly produced food (Driouech *et al.*, 2015).

The findings demonstrate how the course's innovative asynchronous peer-learning methodology serves as a key tool for fostering collaboration among Mediterranean countries. By bringing together participants from diverse cultural and professional backgrounds, this method ensures the effective transfer of essential knowledge and practices. This aligns with the broader perspective that agriculture curricula must be flexible and adaptable to evolving contexts to enhance the effectiveness of future graduates in the workplace.

Furthermore, this training program is foundational in addressing existing gaps in education and fostering the development of sustainable practices in aquaculture. It builds on initiatives such as the distant learning program established by CIHEAM Bari in 2010 to fulfill its mission of education and training (Driouech *et al*., 2015). The study underscores that such approaches are indispensable for equipping the next generation with the expertise needed to ensure sustainable, climate-resilient aquaculture across the Mediterranean region.

## MATERIAL AND METHODS

Two newsletters were written to inform on the opportunity to take part in the online training course with the title “Call for participation | FutureEUAqua Project online Course on “Sustainable, resilient and climate friendly Blue Growth of EU Aquaculture and beyond” | Training program facilitated by CIHEAM Bari | Deadline for application 20 June 2022” (Driouech and Montenegro, 2023).

The call for application was launched in June, on CIHEAM Bari website in which to the participants were asked to provide their name, surname, email address, country of origin, organization or institution/company, position, belonging group (international organization, scientific community (higher education, research), industry/private sector, civil society, NGOs, and others), and stakeholder type.

The course, structured in 4 modules, lasted 6 weeks (from 15th June to 29th July) involving 357 participants coming from 77 countries (Figure 1) mainly from Euro-Mediterranean zone (60%).

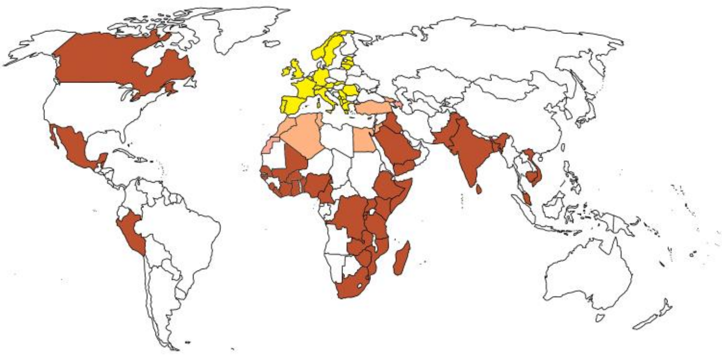

Figure 1. Map of the country participants. In yellow European region, in light orange MENA country and in dark orange other countries

The modules were divided as below:

- Module 0: Introductory eLearning methodology and process
- Module 1: Innovative feeds and feeding strategies for improving welfare & performance of fish in sustainable and organic aquaculture
- Module 2: Consumer perception and preferences regarding aquaculture
- Module 3: Regulatory framework for aquaculture in the EU, with special focus on organic aquaculture

After the course, all statistical parameters were collected such as the learning path (% attend), self-evaluation test, the best score, average time for test attempt (second), forum evidence and interactivity (number of posts) and platform access.

To proof the successful attendance of the course, it is established a threshold value in all the parameters cited above, involving a thoughtful approach to defining performance benchmarks. Begin by clearly outlining the specific objectives and criteria for assessment.

Once the criteria were defined, it was conducted a pilot test with a representative sample to gather data on the distribution of scores. Analyzed the results to determine a baseline and identify the point at which performance can be classified into different levels (e.g., below expectations, meets expectations, exceeds expectations).

Statistical methods such as percentiles, average value and standard deviations are employed to objectively set the threshold. It's essential to strike a balance that is challenging enough to encourage growth but realistic for achievable goals. Regularly review and update the threshold based on ongoing assessments, feedback, and changes in organizational priorities to ensure the self-evaluation test remains a meaningful tool for personal and professional development.

All these data are analyzed and clustered using Excel and R software in order to find a minimum threshold and established those participants deserving to receive a final certificate of attendance and successfully achieved the course, based on the results they achieved.

## RESULTS

The training course was attended by 357 people from 73 countries (Albania, Algeria, Austria, Azerbaijan, Bangladesh, Belgium, Belize, Benin, Burkina Faso, Burundi, Cambodia, Cameroon, Canada, Côte d'Ivoire, Democratic Republic of Congo, Egypt, Estonia, Eswatini, Ethiopia, France, Georgia, Germany, Ghana, Greece, Guinea-Bissau, India, Iraq, Ireland, Italy, Jordan, Kenya, Kosovo, Latvia, Lebanon, Liberia, Lithuania, Macedonia, Madagascar, Malaysia, Mali, Mauritius, Mexico, Montenegro, Morocco, Mozambique, Nigeria, Norway, Pakistan, Palestine, Perù, Portugal, Romania, Rwanda, Saudi Arabia, Senegal, Serbia, Sierra Leone, Slovakia, Somalia, South Africa, Spain, Sri Lanka, Sweden, Switzerland, Tanzania, Tunisia, Turkey, Uganda, United Kingdom, Vietnam, Yemen, Zambia and Zimbabwe).

Thus, the participants' geographic dispersion was: 23% (83 participants) from European countries (geographic Europe), 37% (130 participants) from MENA nations; 40% (144 participants) were foreign nationals, mostly from Africa.

Egypt (42), Morocco (28), Tunisia (25), Nigeria (25), Italy (17), Kenya (16), Ethiopia (12), Algeria (11), Portugal (10), Zimbabwe (9) and Jordan (9), are the top 11 countries in terms of participants.

There were a variety of stakeholders involved, including policy makers (18%), small and medium-sized business managers (32%), innovation brokers (16% of the 59 participants), and large enterprise managers (2%). The remaining 113 participants (32%) were classified as "Other," with 41 of them being researchers, students, PhD candidates, and members of the academic/education community.

A steady portion of participants—45 percent, or 160 people—belonged to the scientific community (research, higher education). Additional categories of membership included: Public Sector 13%, Industry/Private Sector 18%, NGOs 9% (with 33 participants), International Organization 6%, 'Other' 6%, and Civil Society 3%. In the case of belonging groups, the category "Other" was highly variable, making it impossible to identify other subcategories.

Participants were required to complete an assessment form at the conclusion of each module or course to rate the module and its tools. The tool evaluation required participants to rate each tool on a scale of 1 to 5, where 5 represents "very useful" and 1 represents "not at all useful." The module evaluation comprised five categories to be evaluated: fair, good, very good, and exceptional. It is important to note that all the participant-written comments were published in this report exactly as they were written, with no linguistic corrections or modifications.

CIHEAM-Bari assigned a tutor to the group with the following responsibilities: creating the lesson plans, assisting participants in the learning process, facilitating the discussion forum and interactions, answering questions or referring them to outside experts as needed (the system made it possible for any outside expert to access the discussion forum), keeping an eye on any technical issues that arose, gathering and compiling evaluations (Driouech and Montenegro, 2023).

At the end of the course only 47 candidates successfully attended the course. In particular, 16 from European countries, 24 for MENA regions and 7 from other countries (Figure 2). The number of participants and the number of those who achieved successful attendance show a significative difference (T-test=0.036). This is due probably to the dropout phenomena.

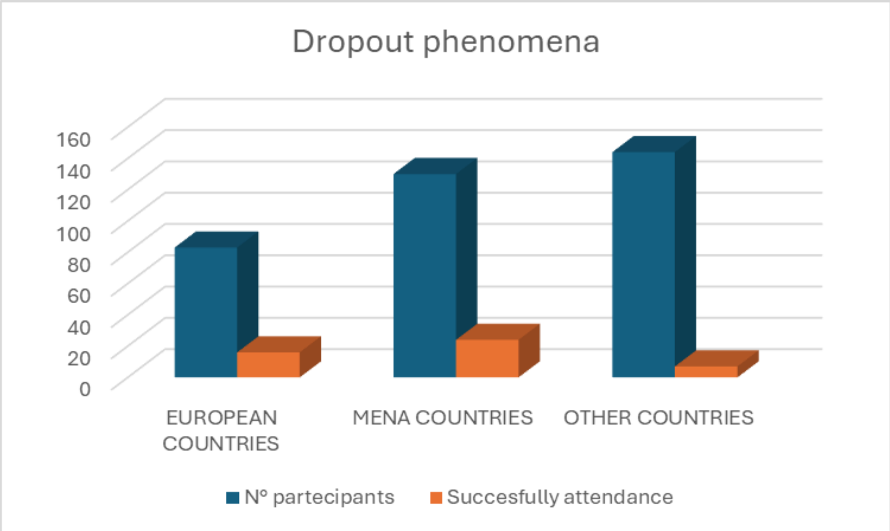


Figure 2. Number of participants (blue) and participants who successfully attendance the course divided for European, MENA and Other Countries

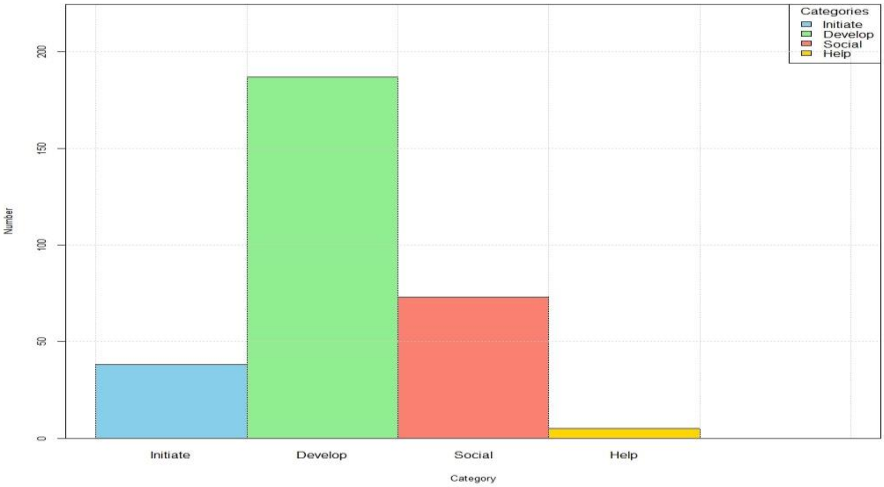


Figure 3. The 4 different types of posts: "Initiate"; "Develop"; " Social / support "and" Help”

This term is commonly used to define a situation where a student leaves his study/activity in which he/she has enrolled before having obtained a formal degree/result.

Dropout can be voluntary according to the student’s perspective.

A quantitative and qualitative analysis of discussions and participants’ various posts and contribution was carried out. In our case, a classification method of Hopkinson (2002) was adopted, which identifies 4 different types of posts: "Initiate"; "Develop"; " Social / support "and" Help” as illustrated in in figure 3).

## DISCUSSION

The growing demand for organic aquaculture has spurred governments to regulate the industry, and standards and certification processes are being developed (Saha 2022).

Despite recent advancements in nutritional studies, the requirements for organic agriculture have only recently been established. In response to these needs, this research seeks to highlight the primary outcomes of the training course aimed at promoting the sustainable growth of aquaculture. The course particularly emphasizes meeting future challenges arising from the escalating consumer demand for high-quality, nutritious, and responsibly produced food. Additionally, discussions encompass feed substitutes and ingredient traceability (Mente *et al.* 2011). Creating new expertise in the sector is essential, and peer learning has emerged as one of the most effective methods for fostering knowledge exchange and skill development. Peer learning enables professionals to share practical insights, experiences, and innovative solutions. Studies such as Smith *et al.* (2020) underscore the role of peer learning in enhancing the efficiency and sustainability of aquaculture practices, facilitating the adoption of advanced technologies, and cultivating a skilled, adaptive workforce that ensures the industry's long-term success.

The asynchronous nature of the course allowed participants to engage flexibly, fostering active collaboration across geographic boundaries. Educational exchanges among students, instructors, and content enhanced engagement and created a sense of belonging within the learning community (Wanstreet, 2006). This collaborative learning framework encouraged participants to formulate questions, discuss problems, explain viewpoints, and develop teamwork skills, directly addressing the sector's multifaceted challenges.

Participants identified several aspects that could improve the course's effectiveness which include:

• Introducing additional tools, such as webinars and social media, to enhance interaction.

• Focusing on fewer topics or a single subject to allow for deeper learning.

• Addressing difficulties with digital tools to ensure inclusivity and accessibility.

Moreover, adult learning principles (Knowles, 1996) play a pivotal role in designing effective training for professionals. Adults prioritize self-directed, goal-oriented learning and bring a wealth of experience that enriches the learning process. Tailoring course content around problem-solving helps maximize engagement and practical application of the knowledge gained. Challenges such as the observed dropout rates also warrant attention. Factors like course timing (during participants' summer vacations) and platform limitations, such as the absence of email notifications for new forum discussions, highlight areas for improvement in future iterations. Addressing these logistical issues will enhance retention and participation.

## CONCLUSIONS

The online course demonstrated its potential to transform research findings into practical knowledge, providing innovative solutions for the aquaculture sector's challenges. It successfully raised awareness within the industry, fostering a community of practice where participants shared their technical and social expertise to implement project outcomes effectively.

Overall, the research highlights the importance of collaborative approaches, particularly peer learning, in developing new professional skills and addressing the sector's needs. By enabling knowledge exchange and fostering a culture of continuous improvement, peer learning has proven to be a valuable strategy for equipping professionals to meet the demands of sustainable aquaculture.

While the course was well-received, key takeaways for future improvements include allocating more resources for course development, updating the e-learning platform, and carefully planning delivery timelines to avoid scheduling conflicts. These adjustments, combined with continued emphasis on collaborative and problem-solving-based learning, will enhance the effectiveness and impact of similar programs in the future.

The CIHEAM Bari course under the FutureEUAqua project serves as a model for how peer learning can catalyze professional development, strengthen international collaboration, and build a resilient workforce in the aquaculture industry.

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# THE FIRST OCCURRENCE OF INVASIVE *Amorpha fruticosa* L. (FALSE INDIGO) IN NORTH MACEDONIA

## SUMMARY

Several specimens of *Amorpha fruticosa* - an invasive alien plant species of North American origin - were recorded in 2020 near the village of Stari Grad, and alongside Babuna river, in the central part of North Macedonia. In the course of a field survey, *A. fruticosa* was found mainly in highly fragmented forests, on forest paths and in forest clearings (coppice), on the edges of arable fields, in a neglected dry meadow, riparian areas and the river banks of Babuna river. In the areas where *A. fruticosa* was detected, it usually grows in association with nitrogen-loving species such as *Chenopodium album*, *Amaranthus retroflexus*, *Calystegia sepium*, *Urtica dioica*, *Sorghum halepense*, and *Rubus caesius*. *A. fruticosa* was found mainly on alluvium and fluvisol soils, predominantly on arable lands along roadsides and at the border between forested areas and arable lands. Control and eradication of *A. fruticosa* is very complicated and costly, mostly due to high reproductive capacity of the species.

**Keywords**: *A. fruticosa*, first occurence, ecological impact, control management

## INTRODUCTION

The genus *Amorpha* L. (*Fabaceae* Juss.: *Amorpheae* Boriss.) contains 16 species, all of North America origin, with a diversity centre in the South-East of the USA (Isely, 1998; Weber and Gut, 2004; Straub *et al*. 2009; CABI, 2019; Grabic *et al*. 2022). Many of the species exhibit a highly restricted geographical distribution. However, the tetraploid *Amorpha fruticosa* L. (false indigo) (2n=4x=40) (Kreuter, 1930; Turner, 1956; Löve, 1982) exhibits “impressive” ecological adaptability (Wilbur, 1975) over its large geographic range, which overlaps that of all of the other species.

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*A. fruticosa* is able to grow on poor degraded, dry and well-drained sandy soils (Doroftei, 2009; Dumitraşcu *et al.* 2011, 2012). This plant can fix air nitrogen, create humus layers and increase soil fertility (Zhao *et al.* 2014), making it suitable for other invasive weeds. In recent years, Kucsicsa *et al.* (2018) reported that *A. fruticosa* is becoming adapted to very humid soils.

In some nature reserves in Romania, large areas invaded by *A. fruticosa* were seen in swamps, beside the forestry paths (Dumitraşcu *et al.* 2013; Dumitraşcu *et al.* 2014). Roadsides are also very important in the spread of this alien species (Kozuharova *et al.* 2020). Additionally, the emergence of *A. fruticosa* downstream from infested sites shows the plant can be spread by water (Fenesi *et al.* 2009). *Amorpha fruticosa* is a perennial, leguminose shrub, 1–3 (4) metres in height with strong woody offshoots. It was introduced to Europe in 1724 (Liovic *et al.* 2002; Karmyzova, 2014) as a plant favoured by beekeepers and for ornamental purposes due to its remarkable dark violet-purplish flowers (Hulina, 1998). Additionally, due to its extensive root system, which expands from horizontal root shoots up to 100 cm below ground, *A. fruticosa* has been utilised for erosion control, for slope stability, and has also been used as a plant to form hedgerows (Celesti-Grapow *et al.* 2009).

In the EPPO region, *A. fruticosa* is listed among the invasive alien plants recognized by the EPPO (EPPO, 2006). It is generally recognized as one of the EPPO regions most invasive alien plant species (Grabic *et al.* 2022), particularly in Central and Eastern Europe where it has been found in Romania (Dumitraşcu *et al.* 2014), Hungary (Varga *et al.* 2016), Slovakia (Kelbel, 2012), Ukraine (Ivanko and Gorban, 2017), Russia (Vinogradova, 2016), Belarus (Euro+Med, 2006), Slovenia (Jogan *et al.* 2012), Croatia (Novak and Novak, 2018), Serbia (Blagojević *et al.* 2015), Bosnia and Herzegovina (Maslo, 2023), and Bulgaria (Pedashenko *et al.* 2012). In Italy, it has become prevalent in northern areas, where it is established in the riverbeds and along the banks of rivers in the Po Plain and the neighbouring valleys (Celesti-Grapow *et al.* 2009).

*A. fruticosa* can survive extreme climatic conditions withstanding freezing temperatures below -25°C in a completely dormant state (Huxley, 1992). It is also very adaptable, being found from the cold continental climate within Northern US to the semi-arid subtropics of Northern Mexico (Huxley, 1992). The plant is adapted to different types of habitats and prefers moist and periodically flooded terrains, such as: river banks, unvegetated or sparsely vegetated shores, water-fringing reed-beds, and riverine (Anastasiu *et al.* 2008), being therefore a serious threat for fragile wet habitats, but can also be adapted to reduced soil moisture, (Dumitraşcu *et al.* 2014) mesophyle and xeromesophyle meadows (Sărăţeanu, 2010). According to Sărăţeanu *et al.* (2008) *A. fruticosa* is mesophytic, and moderate thermophyllic.

*A. fruticosa* is a strong competitor in the banks area and along the channels swamp ecosystem (Doroftei, 2009) and is found in riparian brushes (Anastasiu and Negrean, 2006) and natural riparian forests (Dumitraşcu *et al.* 2011). It may also take place in agricultural areas, but solely in locations where there is sufficient water availability and adjacent to irrigation channels. (Blagojević *et al.* 2015).

Previous studies showed that *A. fruticosa* exhibited vigorous coppicing (Takagi and Hioki, 2013) and demonstrated a significant ability for seed dispersal, rarely by sprouts or layering (Dumitraşcu *et al.* 2014). Sărățeanu *et al.* (2007) demonstrated that grasslands were invaded by *A. fruticosa* due to poor management, large abandoned land offering favorable conditions for its spreading.

*A. fruticosa* propagates by seed, but can also propagate vegetatively by root shoots and plant fragments. Natural spread of propagules can be facilitated by water, small mammals and birds to feed on seeds and contribute to propagation (Doroftei *et al*. 2005). Seeds have a high viability with studies showing seeds gathered from plants growing in sunlight and shadow averaged 94% and 98%, respectively (Oršanić *et al.* 2006). In suitable habitats, viability of seeds in the soil extends from 3 up to 5 years (Stevenson, 2014).

*A. fructicosa* releases some chemical substances that affect the germination of some plant species (Csiszár *et al.* 2013). *A. fructicosa* also tolerates poor soil conditions (Kozuharova *et al.* 2017), characterized through its mutualistic symbiosis with some nitrogen-assimilating bacteria from genus Rhizobium. The bacteria establish nodules on the roots of the shrub, enabling the fixation of atmospheric nitrogen, which is subsequently utilized by *A. fructicosa* and, to some extent, by the surrounding flora, thereby colonizing an environment deficient in nitrogenous compounds (Wang *et al.* 1999; DeHaan *et al.* 2006). *A. fruticosa* fertilizes the soil with nitrogen (Navarrete-Tindall *et al.* 2003), making it suitable for other alien weeds.

*A. fruticosa* has a high capacity to outcompete indigenous plant communities when forming dense monospecific stands. Additionally, it can impact ecosystem services, for example, by obstructing the water flow, consequently increasing the flood risks (Csendes, 2012). In certain regions of Lithuania, *A. fruticosa* can become abundant and invade significant areas of meadow, forest-edge and various other open habitats (Gudžinskas and Žalneravičius, 2015). It has become one of the most harmful weeds in the drainage systems in Croatia (Hulina, 1998).

## MATERIAL AND METHODS

Analyses for the recording of *A. fruticosa* were carried out during the 2020 in the central part of North Macedonia, close to the Stari Grad village (Latitude: 41°34′51″N, Longitude: 21°39′54″E). The first recording was carried out in May and in June a second site visit the population and the wider area was surveyed to investigated the extent of the invasion. Plants were also observed alongside borders of arable fields and neglected dry meadow, riparian areas and the riverbed of Babuna river.

The plant species was identified by using appropriate literature i.e. keys for identification (Kojić, 1981; Domac, 1984; Klapp and Optiz von Beberfeld, 1990). The recording of *A. fruticosa* was done randomly, depending on its spread in different areas.

## RESULTS AND DISCUSSION

In this record, was documented the first occurrence of *A. fruticose* (fig. 1) in the central part of North Macedonia. On 25th of May 2020, while conducting field work in the central part of North Macedonia (fig. 2), and alongside Babuna river (fig. 3), a dense population of *A. fruticosa* - an invasive species in the Macedonian flora - was recorded for the first time in North Macedonia.

Figure 1. *Amorpha fruticosa* L. (False indigo) recorded in the central part of North Macedonia (photograph by Z. Arsov)

Figure 2. Map of Republic of North Macedonia revealing where *A. fruticosa* was detected. The green location is the region where the village Stari Grad is found and where the plants were discovered. The blue places indicate lakes. (https://makedonija.name/municipalities/caska/stari-grad)


Figure 3. Map of Babuna river showing where *A. fruticosa* was found.
(https://www.google.com/maps/@41.63997,21.73484,21398m/data=!3m1!1e3?entry=ttu)

During a second site visit on the 8th of June 2020, the population and the wider area was surveyed to investigated the extent of the invasion. The surveys indicated a significant increase during the intensive flowering stage, accompanied by a population density of *A. fruticosa* that ranged from low to medium. The density of the population was not measured; however, various stands of differing sizes were observed. The largest stands were approximately 100-150 metres at their widest point. During the second survey, the authors extended the area of observation, and the plant was found in moderate and mainly highly fragmented forests growing together with *Salix alba*, *Alnus glutinosa*, and *Ulmus minor*. Further population spread was observed along forest paths and in open spaces in the forest. Plants were also observed to have spread alongside borders of arable fields and neglected dry meadow, riparian areas and the exposed riverbed of Babuna river (fig. 4). The majority of the bushes were under 1 m in width and had several shoots, while only a few individuals exceeded 2 m in width. The roadsides seem to have the essential contribution in encouraging *A. fruticosa* establishment.

Similar to our findings, *A. fruticosa*, according to Hulina (1998) and Karmyzova, (2014), is frequently found in the lowland area, where it is mainly inhabiting riverbanks, drainage systems, forestedges and roadsides. Its spread has been rapid, particularly in disturbed wetland habitats, where it forms dense impermeable monospecific stands destructive for banks, and modifies habitat conditions becoming very competitive regarding autochthonous species (Liebhold *et al.* 2017; Boscutti *et al.* 2020; Pellegrini *et al.* 2021).

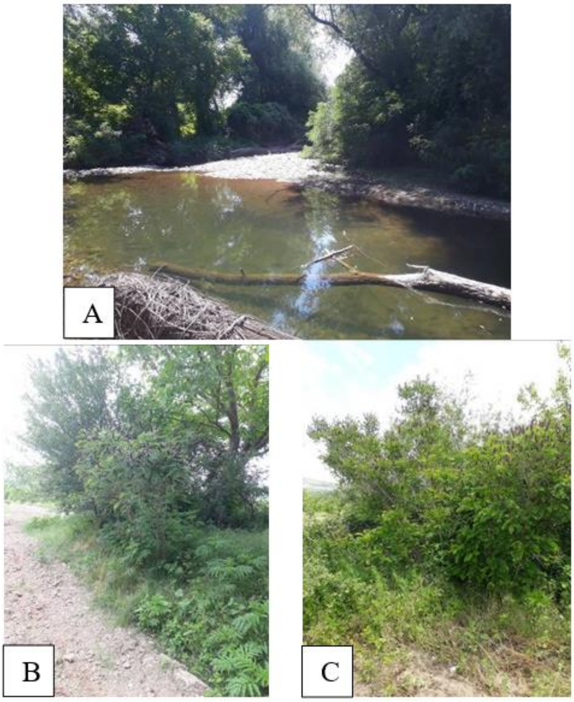


Figure 4. Population of invasive *A. fruticose*, growing in different environments close to the Stari Grad village and alongside Babuna river: (A)-river bank (B and C)-edges of arable land (photographs by Z. Arsov).

*A. fruticosa* demonstrates a preference for fragmented forest environments, indicating that forest fragmentation may heighten the vulnerability of ecosystems to invasive species and contribute to habitat degradation (Turner, 1989). An investigation conducted by (Sărăţeanu, 2010) indicted that *A. fruticosa* enhanced its invasive capacity on grasslands and shrublands. Conversely, dense forests limited dispersion of this heliophilous invasive plant. In this context, Magyar (1960) confirmed that *A. fruticosa* is seen as an inferior forest competitor due to excluding by trees, but because of its rapid growth, shadow superiority and possibly its allelochemical impacts (Elakowich and Wooten, 1995; Xiao *et al*. 2016; Novak *et al*. 2018) and nitrogen-assimilating capability (Wang *et al*. 1999), it is a superior competitor in grasslands (Szigetvári, 2002).

*A. fruticosa* is typically found growing in places where nitrogen-loving weed species including *Rubus caesius* L., *Urtica dioica* L., *Sorghum halepense* (L.) Pers., *Chenopodium album* L., and *Amaranthus retroflexus* L. coexist (Fig. 5). Similar conclusions were reached by Glišić *et al.* (2014) and Radovanović *et al.* (2017), who found a phytocoenological association with the dominance of *A. fruticosa* and *Rubus caesius*, which constitute the most harmful alien plant hotspot and pose a serious threat to native plants and their riverine habitats, including habitats downstream. According to Szigetvári's (2002) research, *A.*

*fruticosa* predominates along with a few other swampy plants and terrestrial creepers, such as *Calystegia sepium*, *Solanum dulcamara*, and the noninative *Echinocystis lobata* in the margins of the areas near to swampy ditches.

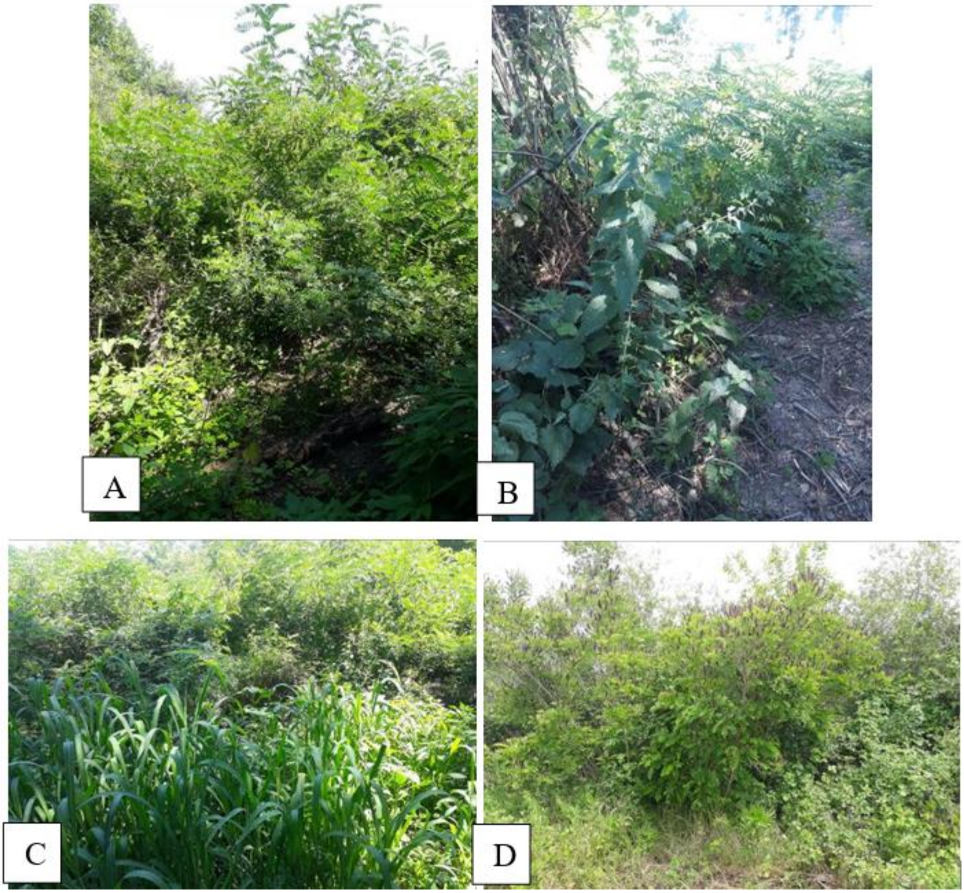


Figure 5. *A. fruticosa* grows in association with nitrogen-loving weed species such as, (A) *Chenopodium album*, *Amaranthus retroflexus*, *Calystegia sepium*, (B) *Urtica dioica*, (C) *Sorghum halepense*, and (D) *Rubus caesius* (photographs by Z. Arsov).

**Ecological impacts**

The most considerable negative consequence of the invasion of *A. fruticosa* is on natural vegetation in floodplains (Nagy *et al*. 2018; Kiss *et al*. 2019). In colonized habitats like swamps, it can have negative impacts on native plant populations and the ecosystem services, e.g. nutrient flow and ecosystem productiveness (Houlahan and Findlay, 2004; Brigić *et al.* 2014). Croatian studies show invaded habitats dominated by *A. fruticosa* are poorer in terms of herbaceous plants, diversity and richness compared to uninvaded habitats (Brigić *et al.* 2014). In several continental regions in Croatia, *A. fruticosa* forms a monodominant community suppressing indigenous vegetation (Novak and Novak, 2018). The grassland species are either unable persist, or only a few persist in conditions of the dense, compact bush formed by *A. fruticosa*. *A. fruticosa* create a physical barrier to waterways and out-compete willow trees that many birds depend on. The environment becomes unsuitable for birds of prey and for the nesting bird species alike (Botta-Dukat and Balogh, 2008). Similarly, the fauna of *A. fruticosa* dominated habitats is impoverished. This kind of bush is nearly impenetrable for large mammals (e.g. deer, for example). These impassable, homogenous *A. fruticosa* stands in floodplains indicate a succession "sink", a particular type of "green desert" (Szigetvari and Toth, 2004). *A. fruticosa* changes indigenous vegetation in damp bushy associations along meadows, pastures, forests, as well as wet habitats, rivers, canals, and swamps. It particularly causes the enormous problems after large expanses in floodplain forests; it will extend very fast, and homogenous stands will be established if no provisions are made. The regeneration of forests in such habitats becomes not possible for comprehensive periods of time (Szigetvári, 2002). It damages the landscape aesthetically in semi-natural habitats, as well, making them impenetrable for visitors (Zavagno and D'Auria, 2001).

### Control management strategies

*A. fruticosa* control management strategies are highlighted by numerous research worldwide, particularly from Asia and Europe (Essl and Rabitsch, 2002; Weber and Gut, 2004; Radulović *et al*. 2008; Takagi and Hioki, 2013; Lončar *et al.* 2020; Kus Veenvliet, 2021), but its control and eradication are complicated and requires substantial resources, due to high reproductive capacity of the species. The methods used are varied, including mechanical techniques, with smaller adverse consequences on the natural environment and surrounding vegetation. But, *A. fruticosa* is defoliation tolerant plant and is able to regenerate to a height of 1 m following annual cutting to 10 cm for period of 7 years (DeHaan *et al.* 2006). Therefore, its eradication by cutting means is labour-intensive. The most promising mechanical control method is the frequent defoliation and digging of the root. In recent years, in the protected areas along the Danube River and other major inland rivers, control measures against the spread of *A. fruticosa* were implemented. The only effective measure against this invasive species proved to be the mechanical removal of the plants followed by replanting native species (Pedashenko *et al.* 2012; Pellegrini *et al.* 2021). For mechanical treatment, the local community was invited to participate, and the branches and roots removed served as fuel wood. Useful initiatives were taken for managers in manufacturing pellets process (Brînzan *et al.* 2020). In order to eradicate *A. fruticosa* from the protected area in Mureș Floodplain Natural Park was mechanically controlled, by repeated cutting of the sprouts of *A. fruticosa*. After selecting the most affected territories by *A. fruticosa*, the shrubs were cut by a forest cutter, and the wood debris was left on the ground to enhance soil quality;

the roots were dislocked by a scarifier machine and they were used by the local communities for heating. The soil was plowed and prepared for sowing with a mix of grasses typically for this region. In order to prevent *A. fruticosa* resettlement, it was advised that control measures should be repeated for at least five years (Suciu *et al.* 2019). When controlled inefficiently, other natural stands, further away from the river meadows, involving partially closed and not too open habitats, may be slowly invaded by *A. fruticosa*.

Different treatment methods for controlling *A. fruticosa* stands, such as manual intervention, flail mowing and grazing by grey cattle was studied by Kapocsi and Danyik, (2015). However, a repeated treatment is necessary in relation to *A. fruticosa*, with costly mechanical flail cutting and grazing. Grazing in forests stands of floodways was possible without diminishing the timber yields, and at the same time it suppresses invasive *A. fruticosa* and has a substantial role in retaining local population as a secondary way of exploitation; coverage ratio of invasive species was diminished due to grazing (*A. fruticosa* non grazed: 50%, grazed 5%) (Varga *et al.* 2016). Mulching had to be repeated annually, which was performed during the autumn and winter periods, while grazing was performed by a cattle herd in cells (a process of managed rotational grazing) (Demeter *et al.* 2021).

Treatment of cut stumps with herbicides provided effective control of *A. fruticosa* (DiTomaso *et al.* 2013). The study of Uzonyi and Miklós (2015) showed that contiguous *A. fruticosa* shrubs were cut with small axes and hatchets, and then incinerated on site. A mixture of red paint, 1 litre of gasoline and 150 mL of triclopyr (Garlon 4E) was applied on the cut stumps, in accordance with nature conservation regulations. Unfortunately, this combination did not provide effective control. Although the stumps were destroyed, the next flood wave spread the seeds all over the cleared area, resulting in 2–3 meter-tall bushes within a year. Polypropylene tree shelters, when used alongside with a reduced rate of herbicides, provided excellent control of *A. fruticosa*. This approach is both cost-effective and environmentally sustainable, while also necessitating minimal human labor (Liovic *et al.* 2002). Also important are policies for the prevention of invasive species, Pötzelsberger *et al*. (2020) concluded that is clear need for more coordinated, science-based policies at the local and international levels is also important to maximize the benefits of non-native trees while mitigating any negative consequences.

## CONCLUSIONS

*Amorpha fruticosa* is likely to continue to spread and invade new areas, especially in habitats that are prone to high levels of disturbance. Additional, future forest fragmentation and clearing, the extension of the transportation network and the abandonment of the agricultural lands will increase the potential spread of *A. fruticose*. Furthermore, planting *A. fruticosa* for different purposes (on the degraded lands, protection of dams or roads) will facilitate species' invasion within the important habitats and ecosystems. To cut twice a year for three years, slowing the vegetative spread and reducing growth and seed production. The cultural control, by offering cover and competition, robust native plant communities will aid in limiting seed germination and preventing the invasion of *A. fruticosa*. Given that *A. fruticosa* is closely associated with certain landscape features, such as soil type, water depth, canopy closure, and road network, it is also critical to incorporate this knowledge into projections of the species' future distribution in habitats and ecosystems that share many of the same environmental conditions. Additionally, these findings can be used to identify the areas that this terrestrial alien species is most likely to colonize by using similar habitats where *A. fruticosa* occurrence is conceivable.

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# AGROECOLOGICAL MONITORING OF WATER ECOSYSTEMS AND SOILS IN THE BASIN OF A SMALL RIVER UNDER THE INFLUENCE OF ANTHROPOGENIC FACTORS

## SUMMARY

In the contemporary socio–ecological–economic life of Ukraine, rural areas hold a special place – they are an integral part of the agro–sphere, as more than a third of the population of our country lives there. These areas are characterized by an exceptional contribution to the formation of the foundations of food security. Increasing the country's export potential makes the development of rural areas one of the main priorities of Ukraine's state policy, aimed at raising the living standards of the rural population, improving the efficiency of the agro–industrial complex (AIC), enhancing the state of the environment, and improving the quality of life of villagers. However, the monitoring of agricultural lands is conducted at the state level, while residential areas where the population grows products for their own needs are rarely studied, and according to the population itself, the norms for the use of chemical protection agents and fertilizers are not always followed. Many settlements in Ukraine with traditional agriculture are located near rivers within watersheds. A modern environmental assessment of rural residential areas located in the basins of small rivers remains relevant, unimplemented, and requires regular research, socio–ecological monitoring, and scientific justification for their ecologically balanced development. One of the important environmental issues is the chemical pollution of water ecosystems in the basin of a small river due to anthropogenic impact. Uncontrolled discharge of wastewater from urban treatment facilities leads to chemical pollution of surface waters within the settlement of Tlumach. The soils of residential areas located

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within watershed basins also experience significant anthropogenic pressure. Agroecological monitoring has been conducted to determine biogenic elements, salt composition, content of trace elements and heavy metals in surface and groundwater, as well as the physico–chemical and agrochemical composition of soils in residential areas within the watershed basin of the Tlumachik River.

**Keywords**: anthropogenic load, monitoring, surface waters, soil, heavy metals, residential areas.

## INTRODUCTION

The issues of environmental safety are relevant for the Western region of Ukraine and are determined by a specific combination of natural, socio–economic, and other factors. Uncontrolled anthropogenic load leads to degradation of agro–landscape, negatively impacting soils, biodiversity, the quality of surface and groundwater, as well as reducing the productivity of agro–ecosystems. (Wilson, 2010; Kanianska, 2016; Litvinova *et al*., 2023a; Litvinova *et al.,* 2023b). Anthropogenic pressure affects the redistribution of elements and substances in the biosphere, leading to the accumulation of their toxic derivatives such as organic substances, phenols, nitrogen and phosphorus compounds, heavy metals in agro–landscapes, particularly in soils and natural waters. The ecological balance between natural and anthropogenically altered landscapes, due to economic activities, is disrupted (Korsun and Palapa, 2014; Palapa *et al.*, 2017; Sasakova *et al.,* 2018; Radchenko *et al.*, 2024a). In the Western region of Ukraine, the main factors influencing environmental safety include: relatively high population density and dense urbanization, waste accumulation, degradation processes linked to both climatic conditions and anthropogenic factors, and outdated and imperfect production technologies, among others (Litvinova *et al.*, 2021). The situation with water supply, drainage, and the quality of drinking water in rural areas is complex. In the regional centers of the Western region, groundwater, surface water, or mixed water sources are used for water supply (Pavlichenko *et al*., 2023; Voitovyk *et al.,* 2023; Havryliuk *et al.,* 2024).

Ukraine is one of the least water–secure countries in Europe, yet water use in the country is predominantly irrational. As a result of toxic, microbiological, and biogenic pollution, the ecological condition of river basins continues to deteriorate steadily (The Law of Ukraine – Document 2697–VIII).

Therefore, continuous monitoring of surface water bodies, implementation of measures for their conservation, restoration, and protection are of utmost importance. Although the region of the Ukrainian Carpathians and adjacent territories are rich in water resources, including river runoff, groundwater, and water in lakes and reservoirs (Prykhodko, 2012), there are many problematic issues related to the environmental safety of these natural sources. Among the main issues are excessive anthropogenic pressure on water bodies due to intensive water management practices, discharge of polluted effluents, leading to increased concentrations of harmful substances in water bodies, and the impact of floods (Kravtsiva, 2013; Andel, 2013). Anthropogenic pressure often leads to the degradation of agro–landscapes, which negatively impacts soils and the quality of surface and groundwater, resulting in the accumulation of organic substances, phenols, nitrogen and phosphorus compounds, as well as heavy metals in them (Wilson, 2010; Kanianska, 2016; Sasakova *et al*., 2018).

The quality of drinking water in centralized and decentralized water supply systems is influenced by the condition of surface water bodies, which serve as water sources (Valerko and Herasymchuk, 2020). The consumption of poor–quality drinking water affects health and leads to increased illnesses in rural populations (Lototska *et al*., 2019). Water quality depends on the geological structure of the land and anthropogenic activities conducted around water bodies, particularly on agricultural waste disposal methods (Mahananda *et al.,* 2010; Davydiuk *et al*., 2020; Shkarivska *et al*., 2021). Overall, the ecological condition of surface waters is influenced by various factors closely related to soil pollution, changes in landscape structure, and disruption of ecologically balanced relationships between fields, meadows, and forests. These factors negatively impact landscape stability, leading to river siltation due to water regime violations in water bodies and land erosion in coastal protection zones (Litvinova *et al*., 2019; Litvinova *et al.*, 2020; Radchenko *et al.*, 2024b).

Other important anthropogenic factors influencing river basins include deforestation, development of degradation processes, land drainage, irrational fertilizer application, improper storage of pesticides in warehouses, creation of artificial reservoirs, canals, channelization of rivers and their tributaries, increased urbanization (settlement) of the basin, and extraction of minerals such as peat, iron ore, oil, and gas (Doroshenko, 2017). One of the sources of surface water pollution is considered to be the private sector, a significant portion of which is not covered by centralized sewage systems, resulting in untreated wastewater being discharged directly into small rivers. Makeshift garbage dumps often appear on the shores of water bodies. The absence of specific land users in nature reserves, the dissolution and fragmentation of collective agricultural enterprises, and their inclusion in the administrative territories of rural and township councils without effective control mechanisms also negatively affect the ecological state of water bodies (Nikolaichuk *et al.*, 2015). Significant challenges to safety and human health arise from water pollution by agricultural enterprises (Jiang *et al.*, 2020).

A critically important issue is the reduction of ecological risk related to the pollution of small rivers (up to 10 km in length, first and second–order streams), which contribute to the flow and water quality of larger rivers. Small rivers are the first and highly vulnerable element (due to their low self–purification potential) of the hydrographic network of a river basin (The Law of Ukraine – Document 2697–VIII). The quality of water in surface water bodies reflects the overall state of the ecosystem. Excessive amounts of harmful substances alter the physico–chemical properties of water, leading to contamination of drinking water sources. Lakes and reservoirs accumulate the highest concentrations of agricultural pollutants. For instance, annually from fields, lakes receive 1554.13 tons of total nitrogen and 1.94 tons of phosphorus, while from meadows, they receive 9.5 tons of nitrogen and 0.20 tons of phosphorus (Česonienė *et al.*, 2021a).

The issue of studying the impact of anthropogenic load on the state of surface waters in the western region of Ukraine is extremely relevant. Equally important are monitoring studies on the condition of naturally sourced drinking water in decentralized water supply systems (wells and pipelines) in rural areas located within river basins. Research on soils within agricultural territories is also crucial, as the population grows and consumes agricultural produce grown there. This necessitates establishing the accumulation patterns of biogenic elements and pollutants within the components of the agro–ecosystem of the Western region of Ukraine in watershed areas.

The aim of the research was to conduct an eco–agrochemical assessment of the state of agricultural landscapes in the western region of Ukraine, including rural areas within the watershed of a small river, and to identify the negative impact of anthropogenic load on the agro–ecosystem.

Agricultural landscapes within watershed basins experience anthropogenic pressure. Considering the complex interaction of factors in modern ecosystems, there is a need to identify environmental issues and determine key indicators for ecologically safe functioning of agricultural landscapes within river basins.

One of the important environmental problems is chemical pollution of water ecosystems in the basin of the small river due to anthropogenic impact. Uncontrolled discharge of wastewater from municipal treatment plants leads to chemical contamination of surface waters within the settlement of Tlumach.

Agroecological monitoring has been conducted to determine the levels of biogenic elements, salt composition, microelements, and heavy metals in surface and groundwater, as well as the physico–chemical and agrochemical composition of soils within the watershed basin of the Tlumachyk River.

For the Ivano–Frankivsk region of Ukraine, significant territorial diversity of soil cover and land resources is characteristic. Almost all agricultural soil groups are found here. In the southeast of the region, there are large areas of chernozems, which are favorable for agriculture. In terms of total surface water resources, Ivano–Frankivsk region ranks third in Ukraine.

Surface waters in the region belong to the Dnister and Prut river basins. The total number of watercourses in the territory of the region exceeds 8,300, with a total length of 15,656 kilometers. Among them, there are 188 rivers longer than 10 kilometers, including 5 rivers that are longer than 100 kilometers – Dnister, Prut, Svicha, Limnytsia та Bystrytsia з Bystrytsia Nadvirnianska (Kravtsiva, 2013). The climate of Ivano–Frankivsk region has a transitional character, ranging from warm and humid Western European to continental Eastern European, with a distinct vertical bioclimatic zoning. The region belongs to the most industrially developed areas of the Western region of the country. The industrial complex of the region includes over 500 large, medium, and small enterprises of various forms of ownership. Main ecological issues related to the development of mineral deposits in the region. In Ivano–Frankivsk region, 94% of the territory is rural. The region has 765 rural settlements, of which 240 or

31% of the total have mountain status. Rural areas are inhabited by 771.3 thousand people (56% of the total population of the region), or 1013 people on average per settlement (compared to 467 people across Ukraine). This indicates that the anthropogenic impact due to agricultural activities on the environment in Ivano–Frankivsk region is also quite significant. The region is located in the zone of the most humid climate in Ukraine (moisture coefficient 1.5–3.0). To increase the efficiency of land use and its conservation, it is necessary to restore the disrupted balance between forests, water bodies, natural fodder lands, and arable land by reducing the latter, that is, by decreasing the amount of land under cultivation. To prevent negative changes in the main components of agroecosystems in Ivano–Frankivsk region, one important direction is conducting agroecological monitoring in the settlement areas within the watershed of small rivers. This helps identify the main factors causing soil contamination, reducing its fertility, and polluting water sources and agricultural produce. It forms the basis for providing recommendations to reduce the negative anthropogenic impact on the ecological condition of rural settlement territories.

## MATERIAL AND METHODS

The research was conducted within the agrolandscapes of Ivano–Frankivsk region, taking into account both stabilizing components (water bodies) and destabilizing components of the ecosystem (agrolandscapes, including agricultural lands - fields, backyard plots). By the method of route monitoring, a survey and sampling of soil and water were conducted in populated areas of Ivano-Frankivsk region within Tlumachka consolidated territorial community (CTC) of Ivano-Frankivsk district. The analysis of natural waters included the Dnister River (Nyzhniv village), Tlumachyk River (right tributary of the Dnister, Tlumach town), right tributaries of the Tlumach River – Dustriv River (Tlumach town) and Mlynivka River (Hrushka village), and left tributaries – Khrust River (Nadorozhna village) and Bzhizhyna River (Tlumach town), as well as ponds (Klubivtsi village, Tlumach town) and springs located in Tlumach town, Lokitka village, Hrushka village, Palahychi village, water from the water intake station (Popeliv village), and soil analysis in Tlumach town, Lokitka village, Klubivtsi village, Melnyky village, and Hrushka village, aimed at determining the direction of migration of biogenic elements and pollutants in the components of the agroecosystem of Tlumachka CTC. Samples of groundwater and soil in the basin of the Tlumachik River were collected in May (Tlumachik River) and August (tributaries of the Tlumachik River) 2022: water – by direct sampling into clean containers, soil – using a soil auger with reference to the sampling locations of water samples.

The Tlumachik River is located within the Tlumachka United Territorial Community of Ivano–Frankivsk district, Ivano–Frankivsk region (Dnister River basin). It is a right tributary of the Dnister River (Black Sea basin). The length of the Tlumachik River is 35 km, with a basin area of 254 $km^2$. The river gradient is 5.7 $m \cdot km^{-1}$. The river valley floodplain V-shaped, with a floodplain width ranging from 50 to 300 m. The riverbed meanders, with a width of 6–7 m and a depth of 1.2 m near the mouth. The river meanders and in places shows signs of a lost channel. It originates on the southeastern outskirts of Hostiv village. It flows predominantly northeast and joins the Dnister River east of Nyzhniv village. The river has right tributaries (Dustriv River, Mlynivka River) and left tributaries (Khrust River, Bzhizhyna River). From its source, the main populated places along its course are Tlumach, Honcharivka, Lokitka, Palahychi, Ostrynia, Oleshiv, Antonivka, and Nyzhniv (Figure 1).

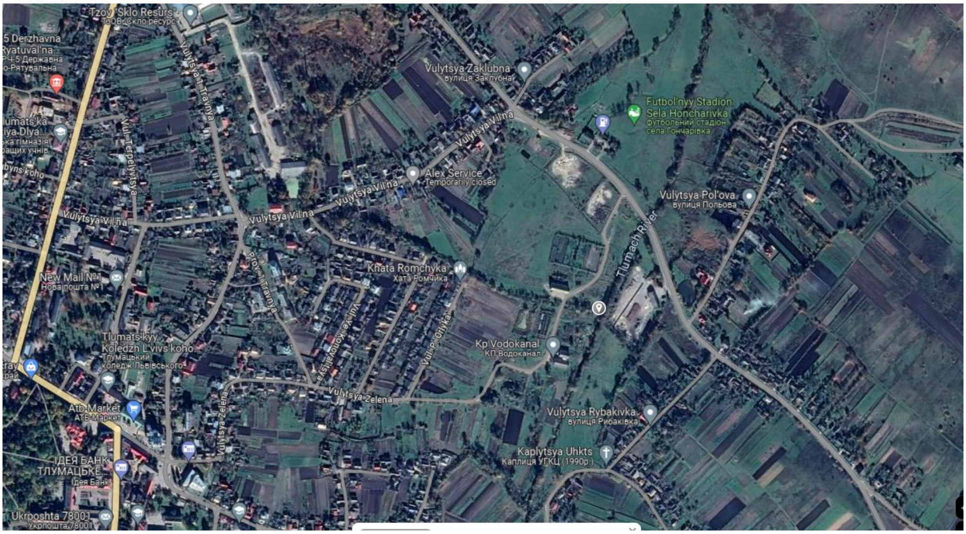

Figure 1. Tlumachik River, Riverbed. Sewage treatment plant (outlet pipe) 48.867655, 25.017406. https://maps.app.goo.gl/iNXf85mSD557HZtn7

In addition to such water quality indicators as acidity according to DSTU 4077-2001, dry residue according to GOST 18164-72, total hardness according to DSTU ISO 6059:2003, the content of various chemical compounds and elements was determined, namely: sulfates according to GOST 4389-72, carbonates and bicarbonates in water extract according to DSTU 7943:2015, nitrates according to GOST 18826-73, phosphorus according to DSTU ISO 6878:2008, chlorides according to DSTU 9297:2007, ammonium according to DSTU ISO 7150-1:2003, potassium according to DSTU ISO 9964-3:2015, as well as copper, manganese, lead, cadmium, zinc, iron, nickel, calcium, magnesium according to GOST 30178-96. In the soil, the following parameters were determined: soil pH (DSTU ISO 10390:2007), hydrolytic acidity (DSTU 7537:2014), organic matter content (humus) (DSTU 4289:2004); light hydrolyzable nitrogen (DSTU 7863:2015); exchangeable potassium and available phosphorus by Kirsanov's method (DSTU 4405:2005), Chirikov's method (DSTU 4115-2002); available compounds of manganese (DSTU 4770.1:2007), available compounds of zinc (DSTU 4770.2:2007), available compounds of cadmium (DSTU 4770.3:2007), available compounds of iron (DSTU 4770.4:2007), available compounds of copper (DSTU 4770.6:2007), available compounds of nickel (DSTU 4770.7:2007), available compounds of lead (DSTU 4770.9:2007); exchangeable calcium and magnesium (DSTU 7861:2015). Chemical-analytical studies of the quality of natural waters and agrochemical studies soil were conducted in the laboratory of the Department of Agroecology and Analytical Research at the Institute of Irrigated Agriculture of the National Academy of Agrarian Sciences of Ukraine, using methods compliant with Ukrainian regulatory standards.

**Statistical analysis**

The least significant difference at $P<0.05$. Statistical processing was performed by Microsoft Excel in combination with XLSTAT.

## RESULTS AND DISCUSSION

The quality of surface water bodies plays an important role in ensuring the social, ecological, and economic well-being of the population living in residential areas. This is why monitoring the condition of surface water bodies is of such great importance. According to Directive 2000/60/EC of the European Parliament and Council of 23 October 2000 establishing a framework for Community action in the field of water policy, Member States must ensure the establishment of water monitoring programs to implement a coherent and comprehensive review of the status of water within each river basin district (Directive of the European Parliament and Council-Document 994_962, 2000). State environmental monitoring of water bodies in Ukraine has a comprehensive approach, but not all water bodies are covered (Shumygai *et al.*, 2021). Climate change, intensive human activity on land, and high plowing of agricultural land are the reasons for the catastrophic destruction of the channels of small rivers, which are becoming shallow, silted, and losing their water flows. There are about 63,000 small rivers in Ukraine, 94% of which have a channel length of up to 10 km. Small rivers are the most vulnerable part of their basins. Their degradation is so evident, but the economic interest of society is the ecological importance of saving small rivers.

Human anthropogenic activity significantly deteriorates the condition of aquatic ecosystems, leading to water quality degradation and threats to human health (Wang and Yang, 2016; Liu *et al*., 2019; Zhao *et al*., 2020; Skyba *et al*., 2021). Due to low water flow and high connectivity with the surrounding land, small rivers are highly vulnerable to changes caused by natural and anthropogenic factors (Ikauniece and Lagzdiņš, 2020).

The issue of the impact of anthropogenic load on the ecological state of natural waters and soil in river basins is studied by many scientists from different countries. According to Loboda and Katynska (2020), the highest values of anthropogenic impact for the Kryvyi Torets River basin (Druzhkivka) correspond to wastewater discharge (point source pollution) and livestock farming (diffuse pollution). Česonienė *et al.* (2021b) note that the largest volumes of pollution in the river basins of Lithuania are caused by the following sources: transboundary pollution, which contributes 87,599 t $ha^{-1}$ $year^{-1}$ of total nitrogen and 5,020 t $ha^{-1}$ $year^{-1}$ of phosphorus; agricultural pollution, which amounts to 56,031 t $ha^{-1}$ $year^{-1}$ of total nitrogen and 2,474 t $ha^{-1}$ $year^{-1}$ of total phosphorus. Zhao *et al.* (2018) indicate that human activities affect nutrient levels in the soil, potentially acting as non-point sources of pollution in the Mun River basin, which is the largest river in northeastern Thailand. Overall, according to researchers, the main sources of pollution within the river basin are wastewater and agricultural activities.

We conducted agroecological monitoring of surface and groundwater, as well as soils of residential areas within the watershed basin of the Tlumachyk River. The small Tlumachyk River, with a watercourse length of up to 30 km, is characteristic of the right-bank tributaries of the Dnister. A comprehensive ecological survey of the Tlumachyk River channel and its four tributaries was carried out, including the determination of chemical indicators of surface and groundwater, and the agrochemical properties of the soils in the Tlumachka territorial community.

The analysis of the water in the Tlumachyk River in the town of Tlumach (sample 2) showed significant pollution near the treatment facilities (pipe outlet) with ammonium nitrogen compounds (7.2 mg·L$^{-1}$), phosphorus (1.4 mg·L$^{-1}$), sulfates (1080 mg·L$^{-1}$), as well as exceeding the indicators for dry residue content (2152 mg·L$^{-1}$), hardness (22 mEq·L$^{-1}$), and calcium (410.5 mg·L$^{-1}$) (Table 1, Figure 2).

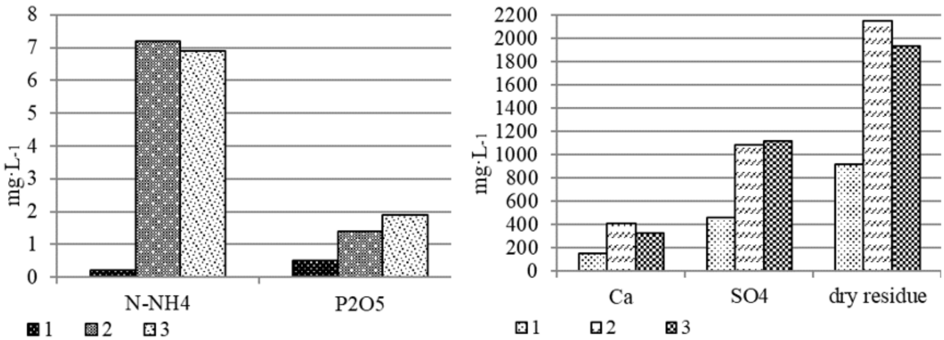


Figure 2. Changes in the content of biogenic elements in river water depending on the distance to the treatment facilities.

Note: 1–Dustryv River, channel 150 m from the treatment facilities, Tlumach; 2–Tlumachyk River, channel, treatment facilities (pipe outlet), Tlumach; 3–Tlumachyk River, 700 m from the treatment facilities, Lokitka village (Ivano-Frankivsk region).

The analysis of the water sampled 700 meters from the treatment facilities in the Tlumachyk River in the village of Lokitka (sample 3) showed a slight decrease in pollution by ammonium nitrogen compounds (6.9 mg·L$^{-1}$), dry residue content (1936 mg·L$^{-1}$), hardness (17 mEq·L$^{-1}$), and calcium (322.7 mg·L$^{-1}$), and even some increase in pollution by phosphorus compounds (1.9 mg·L$^{-1}$) and sulfates (1120 mg·L$^{-1}$).

Table 1. Main indicators of the quality of studied natural waters (rivers) in the territory of Ivano–Frankivsk region (Ivano–Frankivsk district, Tlumachka Unified Territorial Community), 2022

| Place of Sampling | pH (environ-mental reaction) | dry resi-due, mg·L$^{-1}$ | Total Hard-ness, mEq·L$^{-1}$ | Ca | Mg | N-$NO_3$ | N-$NH_4$ | $P_2O_5$ | $K_2O$ | $Na_2O$ | $HCO_3$ | Cl | $SO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mg L$^{-1}$ | | | | | | | | | |
| 1. Dustryv River, 150 meters before the treatment facilities, Tlumach town | 7.2 | 920 | 9 | 146.7 | 21.7 | Traces | 0.2 | 0.5 | 22.4 | 88.4 | 434.3 | 59.6 | 455.0 |
| 2. Tlumachyk River, riverbed, treatment facilities (outlet pipe), Tlumach town | 7.4 | 2152 | 22 | 410.5 | 19.1 | Traces | 7.2 | 1.4 | 9.2 | 28.7 | 346.5 | 45.4 | 1080.0 |
| 3. Tlumachyk River, 700 meters from the treatment facilities, Lokitka village | 7.4 | 1936 | 17 | 322.7 | 17.5 | Traces | 6.9 | 1.9 | 11.2 | 31.2 | 302.5 | 42.6 | 1120.0 |
| 4. Khrust River, 3 km from the source, riverbed, Nadorozhna village | 7.9 | 376 | 4 | 62.5 | 17.5 | Traces | 0.06 | 0.2 | 5.5 | 35.2 | 395.3 | 14.2 | 9.3 |
| 5. Bzhezyna River, estuary, confluence with Tlumachyk River, Tlumach town | 7.4 | 620 | 6 | 103.4 | 14.4 | Traces | 0.04 | 0.6 | 7.3 | 25.5 | 302.6 | 34.1 | 202.0 |
| 6. Mlynivka River, midpoint, 3 km from the source, Hrushka village | 7.6 | 2392 | 22 | 426.9 | 17.4 | Traces | 0.04 | 0.2 | 5.1 | 14.3 | 336.7 | 31.2 | 1560.0 |
| 7. Dniester River, riverbed, Nyzhniv village | 8.0 | 424 | 5 | 84.8 | 12.3 | Traces | Traces | 0.2 | 8.5 | Traces | 253.8 | 48.3 | 196.8 |
| $\overline{X} \pm S\overline{x}$ | *7.5±0.1* | *1260±329* | *12±3* | *222.5±60.1* | *17.1±1.1* | – | *2.06±1.29* | *0.7±0.* | *9.9±2.2* | *31.9±10.4* | *338.8±23.0* | *39.3±5.5* | *660.4±222.9* |
| *V, %* | *3.9* | *69* | *66* | *71.4* | *17.8* | – | *165.0* | *94.4* | *59.8* | *86.6* | *17.9* | *36.9* | *89.3* |
| Least Significant Difference (LSD) 05 | *0.4* | *1139* | *10* | *208.2* | *4.0* | – | *4.46* | *0.9* | *7.7* | *27.6* | *79.7* | *19.0* | *772.5* |
| Quality standards for fisheries water | 6.5–8.5 | 1000 | 7 | 180 | 50 | 9.1 (40 mg $NO_3$ L$^{-1}$) | 0.39 (0.5 mg $NH_4$ L$^{-1}$) | 0.46 (0.2 mg P L$^{-1}$) | 60 (50 mg K L$^{-1}$) | 162 (120 mg Na L$^{-1}$) | Not regulated | 300 | 100 |

Exceedances in manganese and iron levels in the water of the Tlumachyk River were noted, which may be due to natural factors (high background content in the underlying rocks of the Pre-Carpathian region) (Table 2). In the water of the Dustryv River, a tributary of the Tlumachyk River, flowing 150 meters from the treatment facilities in the town of Tlumach (sample 1), the phosphorus content was 0.5 mg·L$^{-1}$, sulfates – 455 mg·L$^{-1}$, and total hardness – 9 mg·L$^{-1}$, exceeding the quality standards for water intended for fisheries but were significantly lower than the water quality indicators in the Tlumachyk River near the treatment facilities. Therefore, the uncontrolled discharge of wastewater from municipal treatment facilities leads to chemical pollution of surface waters within the Tlumach settlement.

Table 2. Main indicators of the quality of studied natural waters (ponds) in the territory of Ivano–Frankivsk region (Ivano–Frankivsk district, Tlumachka Unified Territorial Community), 2022

| Place of Sampling | pH (environ men-tal reac-tion) | dry residue mg·L$^{-1}$ | Total Hardness, mEq·L$^{-1}$ | Ca | Mg | N-$NO_3$ | N-$NH_4$ | $P_2O_5$ | $K_2O$ | $Na_2O$ | $HCO_3$ | Cl | $SO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mg·L$^{-1}$ | | | | | | | | | |
| 1. Pond, 800 meters from the Tlumachyk River, Popeliv village | 7.3 | 2120 | 4 | 60.4 | 8.7 | 1.1 | 0.1 | 0.2 | 2.5 | 12.6 | 200.1 | 36.9 | 40.3 |
| 2. Tlumachka city lake, midpoint of the Bzhezyna River, Tlumach town | 7.6 | 1228 | 3 | 39.8 | 8.8 | Traces | 0.1 | 0.1 | 2.5 | 15.5 | 190.3 | 36.9 | 17.8 |
| 3. Pond (forest), beginning of the Bzhezyna River, Klyubivtsi village | 7.0 | 292 | 3 | 38.4 | 8.5 | Traces | 23.5 | 0.8 | 4.2 | 25.5 | 317.2 | 31.2 | 31.7 |
| $\bar{X} \pm S\bar{x}$ | *7.3± 0.2* | *1213± 528* | *3.3±0.3* | *46.2± 7.1* | *8.7± 0.1* | *0.4± 0.3* | *7.9± 7.8* | *0.4± 0.2* | *3.1± 0.6* | *17.9± 3.9* | *235.9± 40.8* | *35.0± 1.9* | *29.9± 6.5* |
| *V, %* | *4.1* | *75* | *17.3* | *26.7* | *1.8* | *166.3* | *171.0* | *103.2* | *32.0* | *37.9* | *29.9* | *9.4* | *37.9* |
| $LSD_{05}$ | *1.0* | *914* | *2.0* | *43.2* | *0.5* | *2.2* | *47.4* | *1.3* | *3.4* | *23.8* | *247.9* | *11.5* | *39.9* |
| Quality standards for fisheries water | 6.5–8.5 | 1000 | 7 | 180 | 50 | 9.1 (40 mg $NO_3$ L$^{-1}$) | 0.39 (0.5 mg $NH_4$ L$^{-1}$) | 0.46 (0.2 mg P L$^{-1}$) | 60 (50 mg K L$^{-1}$) | 162 (120 mg Na L$^{-1}$) | Not regulated | 300 | 100 |

The water in the Mlynivka River, a tributary of the Tlumachyk River in the village of Hrushka (sample 6), also had significant exceedances in quality indicators such as calcium content (426.90 mg·L$^{-1}$), sulfates (1560 mg·L$^{-1}$), dry residue (2392 mg·L$^{-1}$), and hardness (22 mEq·L$^{-1}$). In the water of this river, an exceedance in copper content (0.01 mg·L$^{-1}$) was also found, indicating the impact of anthropogenic factors. In the water of the Brzezina River, a tributary of the Tlumachyk River in the town of Tlumach (sample 5), exceedances in sulfate content (202.0 mg·L$^{-1}$) and manganese content (0.03 mg·L$^{-1}$) were detected. In the water of the Khrust River tributary in the village of Nadorozhna (sample 4), no exceedance of quality standards was detected, except for manganese content (0.03 mg·L$^{-1}$). In the water of the Dnister River, flowing near the village of

Nyzhniv (sample 7), to which the Tlumachyk River is a tributary, exceedances in sulfate content (156.8 mg·L$^{-1}$) and manganese content (0.03 mg·L$^{-1}$) were also detected. This indicates that pollution by sulfates in the Mlynivka and Brzezina River tributaries, which flow into the Tlumachyk River, as well as in the Tlumachyk River itself, causes these contaminants to enter the Dnister River. Sulfates can be found in detergents, animal waste, fertilizers, pesticides, and naturally occurring minerals in soils, entering groundwater and natural water bodies through natural processes. People who consume water with elevated sulfate levels may experience symptoms such as diarrhea and dehydration. Infants are more sensitive to sulfates than adults. Water containing sulfate levels exceeding 400 mg·L$^{-1}$ should not be used for preparing infant food. Generally, the content of biogenic elements in the tributaries of the Tlumachyk River was found to be lower compared to the quality of surface waters in the Tlumachyk River itself.

The water environment's reaction is an indicator of both natural and anthropogenic origins and varies in river water from nearly neutral to moderately alkaline (pH 7.2–7.9). It is characteristic that increased alkalinity of water is primarily observed in densely populated areas of the Tlumachka community: Khrust River in Nadorozhna village with pH of 7.9, Mlynivka River in Hrushka village with pH of 7.6. Thus, a pattern is identified: the more densely populated the river basin, the higher the alkalinity of the water environment. The study Wang *et al.* (2019) found that changes in pH can significantly impact the productivity of aquatic ecosystems, with acidic water leading to a reduction in productivity and alkaline water leading to an increase in productivity.

Compared to the water of the Dniester River, surface waters of the Tlumachyk River have a less alkaline reaction (pH Dniester River – 8.0, Tlumachyk River – 7.4), significantly higher concentrations of dry residue and sulfates. This indicates that the watershed of the small Tlumachyk River is more vulnerable to intensive pollution by biogenic elements compared to the larger Dniester River. Kim *et al.* (2017) indicate that found that pH plays a critical role in the removal of contaminants, with optimal removal occurring at a pH of 6-8. The study concluded that controlling pH levels is essential for effective contaminant removal from water.

At the time of water sampling, nitrate nitrogen was not detected, while ammonium cation was present in all collected samples ranging from 0.04 to 7.2 mg·L$^{-1}$. As the samples approached agricultural areas, the N-$NH_4$ content increased. The highest levels of ammonium nitrogen, 7.2 mg·L$^{-1}$ and 6.9 mg·L$^{-1}$ respectively, were found in the Tlumachyk River (Tlumach town) near the treatment facilities and 700 meters away in Lokitka village. This indicates direct impact of ammonium pollution in surface waters near densely populated residential areas. In the Dniester River, ammonium nitrogen was only present in trace amounts.

In the Tlumachyk River, 700 meters from the treatment facilities (Lokitka village), excess of the quality standard for fisheries due to nickel content was detected (0.04 mg·L$^{-1}$). In the Dustryv River, 150 meters before the treatment facilities (Tlumach town), zinc content exceeded standards at 0.02 mg·L$^{-1}$, and in the Mlynivka River, at the midpoint 3 km from its source (Hrushka village), copper content exceeded standards at 0.01 mg·L$^{-1}$. This can be attributed to the intensive influence of domestic waste on the presence of these elements in surface waters (Table 3). Heavy metals such as lead and cadmium were not detected in any water sample.

Table 3. Main indicators of quality of investigated natural waters (springs) in Ivano–Frankivsk region (Ivano–Frankivsk district, Tlumachka CTC), 2022

| Place of Sampling | pH (environ-mental reaction) | dry resi-due, mg·L$^{-1}$ | Total Hard-ness, mEq·L$^{-1}$ | Ca | Mg | N-$NO_3$ | N-$NH_4$ | $P_2O_5$ | $K_2O$ | $Na_2O$ | $HCO_3$ | Cl | $SO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mg·L$^{-1}$ | | | | | | | | | |
| 1. Spring 3 meters deep, 10 meters from the Tlumachyk River channel to the treatment facilities, Lokitka village | 7.3 | 936 | 9 | 140.4 | 28.5 | 6.9 | 0.06 | 0.2 | 3.2 | 35.7 | 356.2 | 62.5 | 202.0 |
| 2. Spring 11 meters deep, 400 meters from the Tlumachyk River channel, Lokitka village | 6.9 | 2428 | 27 | 502.4 | 19.2 | 6.8 | 0.1 | 0.2 | 17.2 | 21.0 | 405.0 | 45.4 | 1772.0 |
| 3. Spring 10 meters deep, 1000 meters from the Tlumachyk River channel, Tlumach city | 6.9 | 1412 | 9 | 142.4 | 27.5 | 12.6 | Traces | 0.1 | 1.0 | 29.8 | 341.6 | 82.4 | 75.6 |
| 4. Spring 5 meters deep, 1500 meters from the Tlumachyk River channel, Tlumach city | 7.0 | 860 | 9 | 136.7 | 26.7 | 10.2 | Traces | 0.1 | 0.6 | 39.4 | 351.4 | 59.6 | 276.5 |
| 5. Spring 10 meters deep, 2500 meters from the Tlumachyk River channel, Tlumach city | 7.1 | 360 | 5 | 84.1 | 7.5 | 4.5 | 0.06 | 0.2 | 0.6 | 9.5 | 312.3 | 34.1 | 32.5 |
| 6. Spring 10 meters deep, 80 meters from the Mlynivka River channel, Hrushka village | 6.7 | 104 | 2 | 39.4 | 3.7 | Traces | Traces | 0.1 | 0.6 | 7.6 | 92.7 | 19.9 | 44.5 |
| 7. Spring (active since 1938), 800 meters from the Tlumachyk River channel, Palahychi village | 7.1 | 388 | 5 | 78.4 | 10.8 | Traces | Traces | 1.4 | 19.1 | 40.0 | 375.8 | 45.4 | 45.5 |
| 8. Water intake station, Popeliv village | 7.3 | 1040 | 11 | 195.4 | 15.3 | Traces | Traces | 0.1 | 6.6 | 18.4 | 307.4 | 34.1 | 725.0 |
| $\overline{X} \pm S\overline{x}$ | 7.0±0.1 | 941±260 | 10±3 | 164.9±51.1 | 17.4±3.4 | 5.1±1.7 | 0.04±0.01 | 0.3±0.2 | 6.1±2.7 | 25.2±4.6 | 317.8±34.1 | 47.9±7.0 | 396.7±212.7 |
| *V*, % | 2.9 | 78 | 79 | 87.7 | 55.3 | 94.9 | 92.0 | 149.1 | 126.4 | 51.4 | 30.3 | 41.1 | 151.7 |
| $LSD_{05}$ | 0.2 | 872 | 9 | 171.4 | 11.4 | 5.8 | 0.04 | 0.5 | 9.1 | 15.3 | 114.1 | 23.3 | 713.1 |
| Quality standard for drinking and domestic use | 6.5–8.5 | 1500 | 10 | 130 | 80 | 11.3 (50 mg $NO_3$ L$^{-1}$) | 2.02 (2.6 mg $NH_4$ L$^{-1}$) | Not regu-lated | 24.1 (20 mg K L$^{-1}$)* | 270 (200 mg Na L$^{-1}$)* | Not regu-lated | 350 | 500 |

The monitoring conducted on the model of the Tlumachyk River's surface waters showed that its aquatic ecosystem is vulnerable to environmental disturbances caused by human activities. The river has suffered ecological impact due to the discharge of poorly treated urban wastewater from municipal treatment plants and the influx of biogenic elements from settlements along the river. This scenario sets a precedent for the ecosystem's inability to self-cleanse from excessive harmful substances. The river waters are oversaturated with phosphorus compounds from areas adjacent to the river with treatment facilities and from settlements without proper sewage systems. Phosphorus is considered one of the main elements creating conditions for eutrophication of surface waters. These problems can potentially be addressed through the implementation of a comprehensive ecological program.

In the model of the Tlumachyk River, it is clearly determined that wastewater from treatment plants and settlements near the river's banks, due to the action of organic acids and surfactants contained within them, enhance the influx of predominantly divalent cations such as calcium and magnesium compounds into surface waters. The presence of these compounds determines the concentration of indicators of deterioration that affect water quality. Specifically, there is a direct proportional relationship between the content of dissolved calcium and magnesium ions in water and the content of dry residue, which defines water hardness.

In ecological terms, dry residue serves as a concentrated indicator of water quality, determining the total amount of dissolved mineral inorganic salts including calcium, magnesium, potassium, sodium, heavy metals, and organic substances in the water. Water hardness refers to the saturation of its cations primarily calcium and magnesium–higher concentrations indicate poorer water management properties.

The analysis of water samples collected from a pond located 800 meters away from the Tlumachyk River in Popeliv village (sample 1) and from the Tlumach City Lake (sample 2) showed an excess of dry residue, respectively 2120 and 1228 $mg \cdot L^{-1}$ (Table 2). In the forest pond in Klubivtsi village (sample 3), no excess of dry residue content was noted, but the content of ammonium nitrogen (23.5 $mg \cdot L^{-1}$) and phosphorus (0.8 $mg \cdot L^{-1}$) did not meet the standards, indicating the spread of negative anthropogenic impact not only on ponds located in populated areas but also in natural forest ecosystems. In all ponds, an excess of manganese and iron levels was noted, which may be due to natural factors. Given the natural content of manganese and iron cations in the water bodies of the Tlumachyk River basin, their concentrations reached high levels: 2.8 and 1.67 $mg \cdot L^{-1}$ in the waters of the pond in Popeliv village and 1.27 and 10.1 $mg \cdot L^{-1}$ in the pond in Klubivtsi village, respectively. This is related to the humic nature of the parent rock in these areas and the peat extraction (Table 4). The chemical composition of pond waters, in terms of calcium and magnesium content, shows a tendency for reduced concentrations of biogenic elements compared to the flowing waters of small rivers.

Table 4. Content of trace elements and heavy metals in natural waters (rivers) in the territories of Ivano–Frankivsk region (Ivano–Frankivsk district, Tlumachka CTC), 2022, mg·L$^{-1}$

| Place of Sampling | Cu | Zn | Mn | Fe | Pb | Ni | Cd |
|---|---|---|---|---|---|---|---|
| | mg·L$^{-1}$ | | | | | | |
| 1.River Dustriv, riverbed 150 m to the wastewater treatment plants, Tlumach city | Not detected | 0.02 | 0.22 | 0.25 | Not detected | Not detected | Not detected |
| 2.River Tlumachyk, riverbed, wastewater treatment plants (pipe outlet), Tlumach city | Not detected | Not detected | 0.04 | 0.13 | Not detected | Not detected | Not detected |
| 3.River Tlumachyk, 700 m from the wastewater treatment plants, Lokitka village | Not detected | Not detected | 0.07 | 0.25 | Not detected | 0.04 | Not detected |
| 4.River Khrust, 3 km from the source, riverbed, Nadorozhna village | Not detected | Not detected | 0.03 | 0.06 | Not detected | Not detected | Not detected |
| 5.River Bzhezyna, mouth, confluence with River Tlumachyk, Tlumach city | Not detected | 0.01 | 0.03 | 0.06 | Not detected | Not detected | Not detected |
| 6.River Mlynivka, middle of the river, 3 km from the source, Hrushka village | 0.01 | Not detected | 0.02 | Not detected | Not detected | Not detected | Not detected |
| 7.River Dnister, riverbed, Nyzhniv village | Not detected | Not detected | 0.03 | 0.05 | Not detected | Not detected | Not detected |
| $\overline{X} \pm S\overline{x}$ | – | – | 0.06±0.03 | 0.11±0.04 | – | 0.01±0.01 | – |
| *V*, % | 264.6 | 183.6 | 113.1 | 87.6 | – | 264.6 | – |
| $LSD_{05}$ | – | 0.01 | 0.09 | 0.13 | – | 0.02 | – |
| Norms for the quality of water intended for fisheries | 0.001 | 0.01 | 0.01 | 0.1 | 0.1 | 0.01 | 0.005 |

In water bodies with low flow rates, the lowest water hardness was determined, approaching soft values of 3–4 mg-eq·L$^{-1}$. With a maximum allowable concentration (MAC) of 0.01 mg·L$^{-1}$ for zinc, its content exceeded critical values in the forest pond in Klubivtsi village, reaching 0.03 mg·L$^{-1}$, which is likely related to natural and anthropogenic factors. The content of heavy metals such as copper, lead, and cadmium in the ponds was not detected.

In the water taken from the water supply station (sample 8), there was also an exceedance of the indicators for calcium content (195.4 mg·L$^{-1}$), sulfates (725.4 mg·L$^{-1}$), and hardness (11 mg-eq·L$^{-1}$) (Table 5).

Table 5. Content of Microelements and Heavy Metals in Natural Waters (Ponds) in the Territory of Ivano–Frankivsk Oblast (Ivano–Frankivsk District, Tlumachka CTC), 2022, mg·L$^{-1}$

| Place of Sampling | Cu | Zn | Mn | Fe | Pb | Ni | Cd |
|---|---|---|---|---|---|---|---|
| | mg·L$^{-1}$ | | | | | | |
| 1. Pond, 800 m from the riverbed of the Tlumachyk River, village Popeliv | Not detected | 0.01 | 2.80 | 1.67 | Not detected | 0.01 | Not detected |
| 2. Tlumach City Lake, middle of the Bzhezina River, city Tlumach | Not detected | Not detected | 0.09 | 0.30 | Not detected | Not detected | Not detected |
| 3. Pond (forest), beginning of the Bzhezina River, village Klubivtsi | Not detected | 0.03 | 1.27 | 10.1 | Not detected | Not detected | Not detected |
| $\overline{X} \pm S\overline{x}$ | – | 0.01±0.01 | 1.39±0.78 | 4.02±3.06 | – | – | – |
| *V*, % | – | 114.6 | 97.9 | 131.9 | – | 173.2 | – |
| $LSD_{05}$ | – | 0.05 | 4.77 | 18.6 | – | 0.02 | – |
| Water Quality Standard for Fisheries | 0.001 | 0.01 | 0.01 | 0.1 | 0.1 | 0.01 | 0.005 |

During the expedition, drinking water samples were collected from wells in the Tlumach community. The predominant depth of groundwater is 10 meters, and the distance of the wells from riverbeds ranges from 10–100 meters to 1.0–1.5 kilometers. Groundwater within the Tlumach belongs to non–pressure underground waters, formed as a result of atmospheric precipitation infiltration and water vapor condensation. These waters are generally characterized by a neutral reaction of the water environment but are subject to local contamination depending on the intensity of household activities in the homeowner's yard.

The analysis of water from the well in Lokitka village, located 400 meters from the Tlumachyk River and the sewage treatment facilities, also showed significant exceedances in dry residue content (2428 mg·L$^{-1}$), hardness (27 mg-eq·L$^{-1}$), calcium (502.4 mg·L$^{-1}$), and sulfates (1772 mg·L$^{-1}$) (sample 2) (Figure 3).

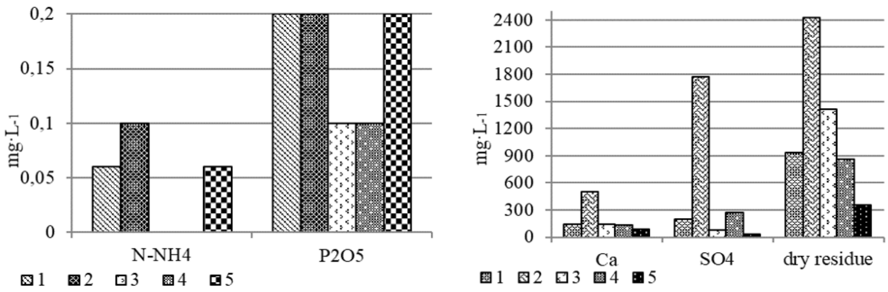


Figure 3. Changes in the content of biogenic elements in well water depending on the distance to the sewage treatment facilities: 1 – 10 meters from the Tlumachyk River to the sewage treatment facilities, Lokitka village; 2 – 400 meters from the Tlumachyk River, Lokitka village; 3 – 1000 meters from the Tlumachyk River, Tlumach town; 4 – 1500 meters from the Tlumachyk River, Tlumach town; 5 – 2500 meters from the Tlumachyk River, Tlumach town (Ivano-Frankivsk region)

In the well (sample 1) located 10 meters from the Tlumachyk River to the sewage treatment facilities, water quality indicators met the standards except for calcium content (140.4 mg·L$^{-1}$). In the well located 1000 meters from the Tlumachyk River in Tlumach (sample 3), water quality indicators showed exceedances in calcium content (142.4 mg·L$^{-1}$), nitrate nitrogen (12.6 mg·L$^{-1}$), and zinc (1.04 mg·L$^{-1}$). In the well located 1500 meters from the Tlumachyk River (sample 4), only the calcium content exceeded the standard (136.7 mg·L$^{-1}$), while in the well located 2500 meters away (sample 5), all indicators met the standards.

Groundwater from wells in the Tlumachyk River basin generally has a neutral reaction of the aqueous solution. Nitrate contamination of drinking water is one of the most significant issues for many regions of Ukraine. Within the Tlumach community, nitrate contamination in well water was practically absent during the summer. The content of ammonium nitrogen was mostly detected in trace amounts. The content of phosphorus, potassium, sodium, and chlorides in the well waters was below the established regulatory values. No exceedances were found for magnesium content in the wells of the Tlumachyk River basin. The presence of lead, cadmium, nickel, copper, manganese, and iron was not detected in the groundwater (Table 6). In one of the wells studied, a slight exceedance of zinc content was found.

Table 6. Content of Microelements and Heavy Metals in Natural Waters (Wells) in the Territory of Ivano–Frankivsk Oblast (Ivano–Frankivsk District, Tlumachka CTC), 2022, mg·L$^{-1}$

| Sampling location | Cu | Zn | Mn | Fe | Pb | Ni | Cd |
|---|---|---|---|---|---|---|---|
| | mg·L$^{-1}$ | | | | | | |
| 1. Well, 3 m deep, 10 m from the riverbed of the Tlumachyk River to the treatment facilities, village Lokitka | Not detected | 0.08 | 0.02 | 0.07 | Not detected | Not detected | Not detected |
| 2. Well, 11 m deep, 400 m from the riverbed of the Tlumachyk River, village Lokitka | 0.01 | 0.31 | Not detected | 0.01 | Not detected | 0.01 | Not detected |
| 3. Well, 10 m deep, 1000 m from the riverbed of the Tlumachyk River, city Tlumach | Not detected | 1.04 | 0.01 | 0.01 | Not detected | 0.01 | Not detected |
| 4. Well, 5 m deep, 1500 m from the riverbed of the Tlumachyk River, city Tlumach | 0.03 | 0.10 | 0.02 | 0.02 | Not detected | Not detected | Not detected |
| 5. Well, 10 m deep, 2500 m from the riverbed of the Tlumachyk River, city Tlumach | Not detected | Not detecte d | 0.02 | 0.04 | Not detected | Not detected | Not detected |
| 6. Well, 10.0 m deep, 80 m from the riverbed of the Mlynivka River, village Hrushka | Not detected | Not detected | 0.03 | 0.13 | Not detected | Not detected | Not detected |
| 7. Well (operating since 1938), 800 m from the riverbed of the Tlumachyk River, village Palagychi | Not detected | 0.01 | 0.04 | 0.01 | Not detected | Not detected | Not detected |
| 8. Water intake station, village Popeliv | Not detected | 0.01 | 0.04 | 0.01 | Not detected | Not detected | Not detected |
| $\overline{X} \pm S\bar{x}$ | 0.01± 0.0 | 0.19± 0.13 | 0.02± 0.0 | 0.04± 0.02 | – | – | – |
| *V, %* | 213.8 | 184.4 | 61.7 | 114.7 | – | 185.2 | – |
| $LSD_{05}$ | 0.01 | 0.42 | 0.02 | 0.05 | – | 0.01 | – |
| Standard for drinking and domestic purposes | 1.0' | 1.0' | 0.5 | 1.0 | 0.01' | 0.02' | 0.001' |

*Note.'– tandards for water supply systems according to State Sanitary Norms and Rules "Hygienic Requirements for Drinking Water Intended for Human Consumption" – 2.2.4-171-10*

Thus, due to anthropogenic impact, pollution of natural waters occurs, which negatively affects the redistribution of elements and substances in the biosphere, leading to the accumulation of harmful compounds in well water at a distance of up to 1000 meters from the pollution source. The water in the well located 80 meters from the Mlynivka River in the village of Hrushka (sample 6) and in the well in operation since 1938 (sample 7) located 800 meters from the Tlumachyk River in the village of Palahychi was within regulatory standards.

Wells in the households of settlements serve as the closest indicators of point-source pollution in the surrounding natural environment, as the intensive use of one's own and neighboring properties directly affects the quality of drinking water through gravitational waters. Household factors include septic tanks, outdoor toilets, animal manure, garbage, and others. In addition, the level of carbonate pollution and water hardness is also determined by the properties of the aquifers.

Considering the results of the obtained studies, it is important to provide recommendations to reduce the negative anthropogenic impact on the ecological state of rural residential areas located within river basins. These recommendations include reducing the doses of manure application, minimizing the use of plant protection products (and if used, being sure to know what and in what quantities), keeping livestock and poultry in special enclosures, and adhering to minimum sanitary protection distances for farm buildings. All of this is possible through agroecological monitoring of rural residential areas, which allows identifying the main factors causing soil pollution.

During the water sampling process, soil samples were also collected for agrochemical evaluation. The soil cover of the Tlumachyk River basin is mainly represented by the following types: dark gray podzolic medium loam soils in the upper reaches of the river, and gray and light gray soils in the middle and lower reaches of the river. As one approaches the Dniester River in the northern part of the region, these soils transition into chernozems, and near the Dniester in the northeast, they transition into meadow chernozem soils. All soil types are highly fertile but have their own characteristics due to genetic traits. The soil cover of the Tlumachyk River basin falls into the category of medium and high fertility based on humus content and nutrient regime, which is associated with the high agronomic quality of the parent rocks, enriched with divalent calcium and magnesium cations.

The analysis of soil taken from a household garden in the village of Lokitka, where the owner applies a lot of organic fertilizers from livestock, showed very high levels of mobile phosphorus and potassium, at 410 and 247.5 $mg \cdot kg^{-1}$, respectively, high calcium content at 19.8 meq/100 g, and elevated magnesium at 2.4 meq/100 g (sample 2) (Tables 7, 8). The water analysis from the well of this household, located 400 meters from the Tlumachyk River and treatment facilities, also showed significant exceedances in indicators of dry residue content (2428 $mg \cdot L^{-1}$), hardness (27 $meq \cdot L^{-1}$), calcium (502.4 $mg \cdot L^{-1}$), and sulfates (1772 $mg \cdot L^{-1}$) (sample 4). In the garden located on the property in the city of Tlumach, very high levels of mobile phosphorus (462.5 $mg \cdot kg^{-1}$) and high levels of potassium (132.5 $mg \cdot kg^{-1}$) were also noted. However, the water analysis from the well located 2500 meters from the Tlumachyk River treatment facilities showed that all indicators met the standards. Therefore, keeping a significant number of livestock on the property, uncontrolled application of organic fertilizers to the household plot, and the short distance to the treatment facilities could have caused excessive amounts of phosphorus and potassium in the soil and contamination of the well water of the household owner who keeps a significant number of livestock.

In the village of Hrushka, 15 meters from the left bank of the Mlynivka River on a field for grazing poultry (sample 5), the analyzed soil samples showed elevated levels of easily hydrolyzable nitrogen (231.1 mg·kg$^{-1}$), very high levels of mobile phosphorus (288.5 mg·kg$^{-1}$), elevated potassium (117.5 mg·kg$^{-1}$), and very high levels of calcium (51.8 meq/100 g), which may be due to the large amount of bird droppings.

Table 7. The physical–chemical and agrochemical condition of soils in the territory of Ivano–Frankivsk region (Ivano–Frankivsk district, Tlumachka united territorial community), layer 0–20 cm, 2022

| № | Place of Sampling | Exchangeable Acidity, pHsol. | Hydrolytic Acidity, meq/100 g | Organic Matter Content Recalculated to Humus, % | Easily Hydrolyzed Nitrogen, N | Available Phosphorus, $P_2O_5$ | Available Potassium, $K_2O$ |
|---|---|---|---|---|---|---|---|
| | | | | | meq/100 g | | |
| 1 | Tlumach city, 20 meters from the right bank (treatment facilities), Tlumachyk River. | 6.6 | 0.87 | 5.03 | 196.0 | 80.0 | 270.0 |
| 2 | Lokitka village, private property, garden (where a lot of organic fertilizers from livestock are applied). | 6.5 | 0.64 | 3.62 | 117.6 | 410.0 | 247.5 |
| 3 | Klubivtsi village, 20 meters from the lake, field (winter rapeseed). | 4.6 | 4.52 | 3.58 | 140.0 | 53.0* | 109.5* |
| 4 | Melnyky village, 100 meters from the lake, on the left side of the dam, field (winter wheat). | 5.4 | 2.02 | 4.15 | 140.0 | 142.5 | 247.5 |
| 5 | Hrushka village, 15 meters from the left bank of the Mlynivka River, field (for grazing poultry), 3 kilometers from the source. | 7.1 | 0.93 | 5.78 | 231.1 | 288.5 | 117.5 |
| 6 | Tlumach city, Vavilova Street 9, garden, private property. | 7.0 | 0.49 | 2.45 | 81.2 | 462.5 | 132.5 |
| | $\bar{X} \pm S\bar{x}$ | *6.2±0.4* | 6.2±0.4 | 1.58±0.63 | 4.10±0.48 | 150.9±22.1 | 276.7±67.4 |
| | *V, %* | 15.9 | 29.4 | | | | |
| | $LSD_{05}$ | 1.5 | 15.9 | 97.5 | 28.7 | 35.9 | 59.7 |

The analysis of soil samples taken from fields in Klubivtsi village (winter rapeseed) and Melnyky village (winter wheat) indicated increased soil acidity and decreased fertility compared to soil samples taken from household gardens. In the soil sampled in Tlumach city, located 20 meters from the Tlumachyk River treatment facilities, moderate levels of easily hydrolyzable nitrogen (196 mg·kg$^{-1}$) and very high levels of mobile potassium (270 mg·kg$^{-1}$) were observed.

Table 8. The content of microelements, heavy metals, calcium, and magnesium in soils in the territory of Ivano–Frankivsk region (Ivano–Frankivsk district, Tlumachka united territorial community), layer 0–20 cm, 2022

| Sample | Place of Sampling | Cu | Zn | Pb | Ni | Cd | Mn | Fe | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mobile forms (extracted with acetate-ammonium buffer solution pH 4.8), $mg\cdot kg^{-1}$ | | | | | | | meq/100 g | |
| 1 | Tlumach city, 20 meters from the right bank (treatment facilities), Tlumachyk River | 0.42 | 1.6 | 1.7 | 1.1 | 0.15 | 16.8 | 6.2 | 21.6 | 2.1 |
| 2 | Lokitka village, private property, garden (where a lot of organic fertilizers from livestock are applied) | 0.12 | 1.3 | 1.0 | 0.8 | 0.15 | 17.8 | 0.8 | 19.8 | 2.4 |
| 3 | Klubivtsi village, 20 meters from the lake, field (winter rapeseed). | 0.21 | 1.0 | 1.4 | 2.2 | 0.16 | 40.1 | 40.2 | 12.7 | 2.2 |
| 4 | Melnyky village, 100 meters from the lake, on the left side of the dam, field (winter wheat) | 0.18 | 0.8 | 1.2 | 0.9 | 0.12 | 15.7 | 3.7 | 57.9 | 2.1 |
| 5 | Hrushka village, 15 meters from the left bank of the Mlynivka River, field (for grazing poultry), 3 kilometers from the source | 0.54 | 2.3 | 3.1 | 1.2 | 0.22 | 20.1 | 15.9 | 51.8 | 2.0 |
| 6 | Tlumach city, Vavilova Street 9, garden, private property. | 0.22 | 2.0 | 1.8 | 0.7 | 0.15 | 13.0 | 1.4 | 24.3 | 2.3 |
| | $\overline{X} \pm S\overline{x}$ | *0.28± 0.07* | *1.5± 0.2* | *1.7± 0.3* | *1.1± 0.2* | *0.16± 0.01* | *20.6± 4.02* | *20.6± 4.0* | *11.4± 6.2* | *2.2± 0.1* |
| | *V, %* | *57.5* | *38.6* | *44.0* | *47.5* | *20.9* | *47.8* | *47.8* | *133.3* | *6.7* |
| | $LSD_{05}$ | *0.24* | *0.9* | *1.1* | *0.8* | *0.05* | *14.6* | *14.6* | *22.5* | *0.2* |
| | Maximum permissible concentration (MPC) | | | | | | | | | |
| | | 3 | 23 | 6 | 4 | 0.7 | – | – | – | – |

Overall, soils sampled in the Tlumachyk River basin had a neutral reaction, with an average humus content of 4.6%. In specific locations, particularly in Hrushka village (Mlynivka River), Tlumach city (mouth of the Solonyk River), Melnyky village (mouth of the Solonyk and Mlynivka rivers), the humus content was high, ranging from 5.8% to 8.5%. The average content of easily hydrolyzable nitrogen was 161 $mg\cdot kg^{-1}$, indicating an elevated supply level. The mobile phosphorus content was 210 $mg\cdot kg^{-1}$, and potassium was 229 $mg\cdot kg^{-1}$, both indicating high to very high supply levels with significant variability. The content of microelements and heavy metals did not exceed the maximum permissible concentration (MPC) values.

Thus, the analysis of soil samples in the Tlumachyk, Mlynivka, and Bzhizhyna River basins indicates a sanitary-safe level of heavy metal contamination in the arable layer, except for isolated cases in areas with high anthropogenic loads. This will enable the designation of areas for organic farming and livestock as profitable sectors of agricultural production in the future. However, about 30% of the soil cover is eroded, significant areas are unproductive with shrub overgrowth, and grasslands are low-yielding, requiring a systemic approach to the restoration of the Tlumachyk River basin.

Due to the fact that the ecological condition of suburban areas often does not meet sanitary and hygienic standards and rules, which is associated with small areas of backyard plots that are often not maintained due to lack of knowledge and awareness among rural populations, it is necessary to implement education on ecological issues in rural communities. This education should cover potential problems arising from uncontrolled use of organic and mineral fertilizers, pesticides and plant disease control agents, livestock and poultry management, storage of manure, and failure to maintain adequate distances from water supply sources to outbuildings, privies, compost pits, and garbage bins on backyard plots.

As a result of anthropogenic influence, pollution of natural waters and soil occurs in suburban areas, negatively affecting the redistribution of elements and substances in the biosphere, leading to accumulation of compounds such as calcium, sulfates, significant exceedance of dry residue and hardness indicators in well water within 400 meters from the pollution source, and excessive accumulation of available phosphorus and potassium in the soil. To improve the quality of surface and groundwater and prevent their chemical pollution, it is necessary to construct modern standardized treatment facilities within the settlements of the small river basin and establish centralized sanitary–hygienic purification systems.

## CONCLUSIONS

1. Agroecological survey of the Tlumachyk River basin in the Tlumachska amalgamated territorial community (CTC) of Ivano-Frankivsk district, Ivano-Frankivsk region, was conducted. The basin stretches for 35 km with an area of 254 km², including 4 tributaries: the right ones are Dustriv and Mlynivka, and the left ones are Khrust and Bzhezyna. Tlumachska OTG comprises 18,099 residents across 34 villages, focusing on agricultural activities.

2. Two field expeditions were conducted for reconnaissance and sampling of surface and subsurface water, ponds, and soil in the floodplain of the Tlumachyk River to determine agrochemical characteristics, cation composition of macro– and microelements, and heavy metals for the agroecological assessment of the river basin.

3. The chemical composition determination of surface waters collected from the Tlumachyk River revealed a direct correlation between the increase in concentration of dry residue, ammonium nitrogen, total phosphorus and potassium forms, sodium, bicarbonates, chlorides, and sulfates with the intensity of anthropogenic influence on water quality. These elements serve as indicators of chemical pollution by biogenic elements due to their high concentrations. The highest degree of surface water pollution was identified near the urban sewage treatment plants of Tlumachyk and settlements located in protective zones along the river banks. Nearly 70% of water samples showed elevated concentrations of phosphorus compounds, increased water hardness, carbonate content, and levels of iron, manganese, and nickel, which increase with increasing anthropogenic load. Copper, zinc, lead, and cadmium content in Tlumachyk River waters are almost absent. Generally, the content of biogenic elements in the Tlumachyk River tributaries was lower compared to the quality of surface waters in the Tlumachyk River itself.

3.1. Monitoring conducted on a model of the Tlumachyk River's surface waters showed that its aquatic ecosystem is vulnerable to environmental disturbances caused by human activities. The river has suffered an ecological impact due to the discharge of poorly treated sewage from urban sewage treatment plants and the influx of biogenic elements from settlements along the river. This creates a precedent of the ecosystem's inability to self-cleanse from an excess of harmful substances. The river waters are oversaturated with phosphorus compounds in areas adjacent to sewage treatment facilities and settlements without proper sewage systems.

3.2. In the forest pond in the village of Klubivtsi, excess levels of ammonium nitrogen (23.5 mg·L$^{-1}$) and phosphorus (0.8 mg·L$^{-1}$) indicate the spread of negative anthropogenic influence not only on ponds located in areas directly inhabited by the population but also in natural forest ecosystems.

3.3. All investigated ponds show excess levels of manganese and iron, which may be due to natural factors. The chemical composition of pond waters in terms of calcium and magnesium tends to lower the concentration of biogenic elements compared to the flow waters of small rivers.

3.4. Due to the low flow rate of water in the ponds, they have the lowest water hardness, approaching soft values of 3–4 mg-eq·L$^{-1}$. Zinc content exceeded critical levels in the forest pond in the village of Klubivtsi, reaching 0.03 mg·L$^{-1}$, which is evidently linked to natural and anthropogenic factors. The presence of heavy metals such as copper, lead, and cadmium in the ponds was not detected.

4. Groundwater from springs in the basin of the Tlumachyk River mostly has a neutral pH. Within the Tlumachka region, nitrate pollution in spring waters during summer was practically absent. Ammonium nitrogen content was mostly trace amounts. Phosphorus, potassium, sodium, and chloride levels in spring waters were below regulatory limits. Magnesium content in the springs of the Tlumachyk basin did not exceed permissible levels. Lead, cadmium, nickel, copper, manganese, and iron were not detected in groundwater.

4.1. A spring located 400 m from the Tlumachyk River and sewage treatment facilities (village of Lokitka) showed significant exceedances in dry residue content (2428 mg·L$^{-1}$), hardness (27 mg-eq·L$^{-1}$), calcium (502.4 mg·L$^{-1}$), and sulfates (1772 mg·L$^{-1}$). At a distance of 1000 m from the Tlumachyk River in Tlumach town, water quality indicators in the spring exceeded limits for calcium (142.4 mg·L$^{-1}$), nitrate nitrogen (12.6 mg·L$^{-1}$), and zinc (1.04 mg·L$^{-1}$). At 1500 m from the river, only calcium content exceeded the standard (136.7 mg·L$^{-1}$), while at 2500 m, all indicators met regulatory norms. This indicates that anthropogenic influence leads to pollution of natural waters, adversely affecting the distribution of elements and substances in the biosphere, resulting in the accumulation of harmful substances in well water within 1000 m of the pollution source.

5. Agronomic monitoring of rural settlement areas reveals the main factors contributing to soil contamination. Based on the research results, reducing the application rates of manure, minimizing the use of plant protection products, and maintaining agricultural grasslands and poultry in designated areas will contribute to improving the ecological situation of rural settlement areas.

5.1. The soil cover of the Tlumachyk River basin, as determined from collected samples, exhibits a neutral reaction, with an average humus content of 4.6%. In specific locations, humus content is high, ranging from 5.8% to 8.5% (in villages such as Hrushka, along the Solonyk River mouth in Tlumach, and in Melnyky near the Solonyk and Mlynivka rivers). The average content of hydrolyzable nitrogen is 161 $mg \cdot kg^{-1}$, indicating an elevated level of availability. Available phosphorus levels are 210 $mg \cdot kg^{-1}$, and potassium levels are 229 $mg \cdot kg^{-1}$, indicating high to very high availability. The content of microelements such as copper, zinc, manganese, and nickel in the soil samples collected is below the sanitary and hygienic standards (GDK), as is the content of heavy metals.

5.2. Overall, the soil cover is characterized as potentially highly fertile, with areas that could potentially be suitable for organic farming. However, approximately 30% of the soil cover has been eroded, with significant areas being unproductive, overgrown with shrubs, and low-yielding grasslands, which requires a systematic approach to the basin's restoration issues along the Tlumachyk River.

In general, the basin of the Tlumachyk River can be classified as an ecologically balanced region with local manifestations of environmental stress in areas of high anthropogenic pressure. These areas include the vicinity of wastewater treatment plants at the headwaters of the Tlumachyk River, settlements located directly along the riverbank, tributaries of the Tlumachyk River, and ponds. Restoring the Tlumachyk River basin requires a complex of engineering and cultural-technical measures to restore watercourses and fisheries, as well as to bring the sanitary–hygienic conditions of the community in line with environmental standards.

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***Milica MARČETA*[1], Ljiljana Keča[2]***

# THE ROLE OF FOREST RESOURCES IN THE DEVELOPMENT OF SOUTHEASTERN SERBIA: MARKET ANALYSIS AND PERSPECTIVES

## SUMMARY

The global wood trade is important for the world economy and significantly impacts the construction, paper, and bioenergy industries. Growing demand comes from industrialized countries and developing economies, which invest in urbanization and infrastructure development. As a large consumer and wood exporter, Europe is influenced by climate change, geopolitics, and energy crises. On the other hand, Serbia has a long tradition in wood export, but at the same time faces challenges in modernization and sustainable forest management. In such circumstances, this research aims to analyze the market trends in wood assortments in the Southeastern Forest Area of Serbia in the period 2008-2017. The purpose of the research is to identify the main trends in the field of logging, processing, and sales of wood assortments in the analyzed area. The subject of the research includes state forests, Public Enterprise (PE) "Srbijašume", forest estates in the Southeastern Forest Area, the volume of logging, production, and sales of wood assortments, as well as their price.

The data were collected from the databases of the PE "Srbijašume" and included annual sales and prices of beech, oak, and poplar, which were adjusted according to annual price indices to calculate their real prices. The methods used in the analysis include descriptive statistics, the Mann-Kendall test for trend detection, and autocorrelation of time series.

The results indicate a statistically significant increase in the volume of felling, sales, average prices, and gross revenues during the analyzed period. Beech and oak dominate the sales, reflecting their importance in the regional forest inventory. This data confirms the steady growth of the wood industry sector in the South East region and underlines the importance of responsible resource management and adaptability to market dynamics.

**Keywords:** wood market, Southeast Serbia, PE "Srbijašume", logging, sales

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## INTRODUCTION

In general, the wood trade is an important component of the world economy and has a significant impact on industrial sectors such as construction, furniture, paper, and bioenergy. The growing demand for wood and wood products comes from economically developed and developing countries that are intensively investing in urbanization and infrastructure.

Changes strongly influence global wood markets in a wide range of regulatory and market factors (Latta *et al.,* 2016), in particular: population, economic growth, technological changes, the environment, etc. (de Fégely 2005; 2009). The main exporters of wood products are Canada, Russia, the USA, and the Scandinavian countries, which have large forest resources and developed infrastructure for processing and export. On the other hand, importers are often from countries with high urbanization levels and construction activity, such as China, Japan, and India. Sustainable forest management has become key to regulating trade and protecting forests, and some countries are implementing eco-labeling and certification to meet environmental standards.

The European wood market and wood trade are affected by several major factors, such as global climate change – through the increasing volume of sanitary logging, the global economic crisis manifested in the reduction of wood product consumption, fossil fuel prices, the geopolitical situation, the volume of wood supply on the market, etc. (Gejdoš, Potkány, 2017; Stare, Ščap, 2019).

In Serbia, the wood trade has a long tradition with a significant share in the national economy and an important role in rural areas. Serbia's main export partners in this area are European Union countries, including Germany, Italy, Slovenia, and Romania. The European Union already plays a vital role in trade with the Western Balkans and in further integrating the Western Balkans into the economic and political structures of the European region (Zdrahal *et al.,* 2024).

Problems with illegal logging and the need to modernize the processing sector and forest management pose challenges to sustainable development and wood trade in Serbia. The focus is increasingly on the implementation of sustainability certifications (such as FSC and PEFC) to encourage environmentally responsible production and increase export competitiveness. These standards have become the most diffused standards at the global level (Lombardo, 2024).

Based on the situation in Serbia, where supply and demand for raw materials are not matched, public enterprises (PEs) play an important role in their distribution (Praščević, 2015). In this sense, the pre-defined criteria for priority purchases are respected, which are, in the case of technical wood:

-buyers who have their own installed wood processing capacities;

-number of wood processing stages performed by the buyer;

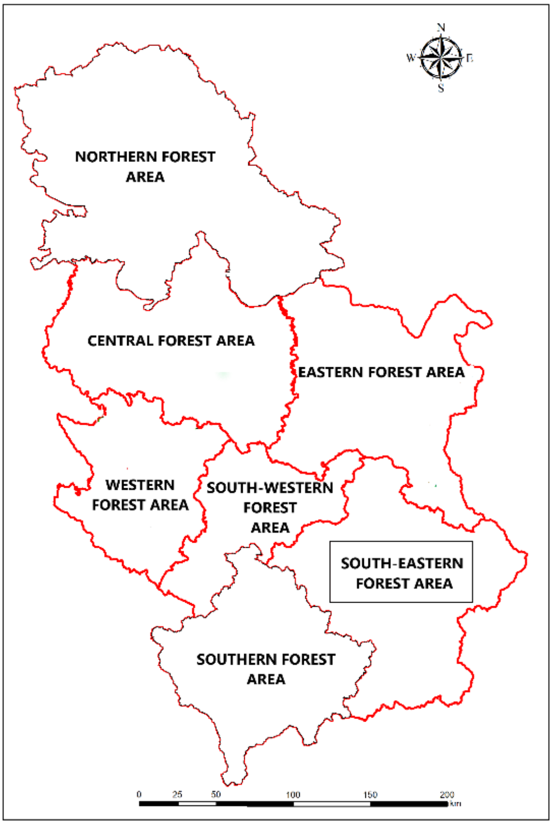
-compliance with previous contractual obligations, a buyer who has complied with contractual obligations in the current year will be offered approximately the same quantity in the following year as he took over in the previous year;

-level of development of the buyer's municipality, i.e. processing plants with the majority of employees (Glavonjić *et al.*, 2016).

The aim of the research is to examine market trends and potential in the Southeastern Forest Area. The purpose is to identify trends in logging, production, and sale of wood assortments in the analyzed area. The subject of the research is state forests, PE "Srbijašume" and forest estates in the Southeastern Forest Area, the volume of logging (production), sale of wood assortments, and their prices.

## MATERIAL AND METHODS

The data used in this article were generated from internal databases and reports of the PE "Srbijašume". Specifically, the annual data on sales (in m³) and wood prices (in dinars) for beech, oak, and poplar in the Southeastern Forest Area were analyzed. Nominal wood prices were adjusted using annual price indices to achieve comparability over the observed period. This calculated the real price of wood. Based on the real price and sales volume, the annual gross income for each of the three wood types was calculated (Marčeta, Keča, 2024).




Map 1. Research area

The research area refers to the Southeastern Forest Area, which includes the forest estates of Leskovac, Niš, Vranje, Kuršumlija, and Pirot (Map 1). The period analyzed is 2008-2017.

Based on the data, time series were formed and in the first phase, significant autocorrelations (dependence within the data) were examined using the autocorrelation coefficient graph (Wang, 2008). Autocorrelation was calculated in two steps. In the first step, the dependence between data of consecutive years was examined, for step 2, the dependence between data every two years, etc. For the autocorrelation coefficients, the confidence interval (95%) is indicated on the graph. If the autocorrelation coefficient for any of the steps is outside the confidence interval, it is considered that there is a statistically significant dependence of the data with that step. The significance of the trend was tested using the Mann-Kendall test (Mann, 1945; Kendall, 1975; Kulkarni, von Storch, 1995; Yue, Wang, 2004). This method is most commonly used for trend detection, the non-parametric Mann-Kendall test (Hamed, Rao, 1998; Ghalharia *et al.,* 2012; Guhathakurta *et al.,* 2010), which assumes the independence of data in the time series (Yue, Wang 2004; Pohlert, 2016).

## RESULTS AND DISCUSSION

Table 1 presents total logging, sales, gross revenue, as well as the average price and standard deviation of the price in the Southeastern Forest Area, in the period 2008-2017.

Table 1. Descriptive statistics

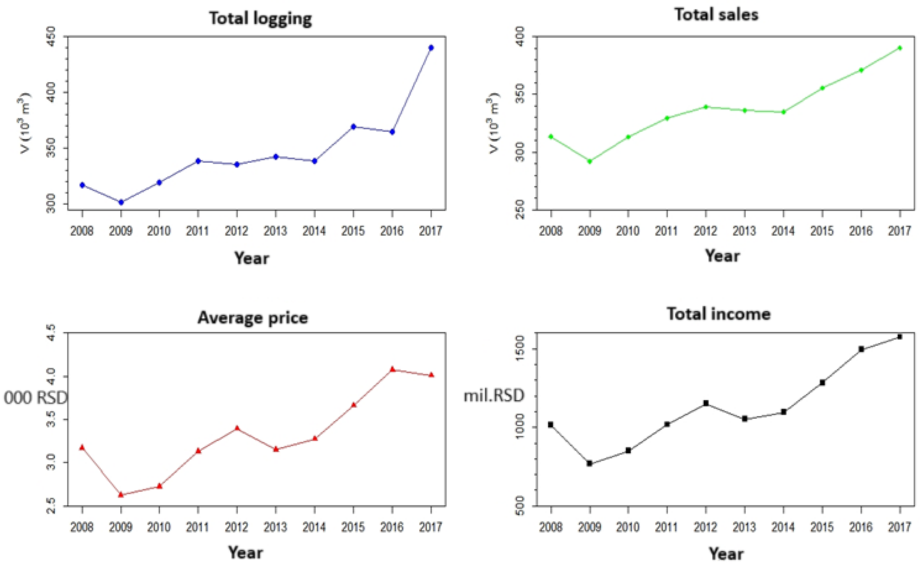
| YEAR | LOGGING | | SALES | | PRICE | | GROSS INCOME | |
|---|---|---|---|---|---|---|---|---|
| | TOTAL ($m^3$) | % | TOTAL ($m^3$) | % | Average value (RSD) | Standard deviation | TOTAL (RSD) | % |
| 2008 | 316,795 | 28.1 | 313,422 | 28.8 | 3,172.2 | 636.9 | 1,013,365,655 | 33.8 |
| 2009 | 301,703 | 27.3 | 292,039 | 27.4 | 2,622.4 | 344.0 | 768,532,387 | 25.8 |
| 2010 | 319,199 | 27.3 | 313,185 | 27.4 | 2,722.2 | 309.6 | 850,522,283 | 23.8 |
| 2011 | 338,912 | 27.9 | 329,268 | 28.5 | 3,130.0 | 286.9 | 1,018,114,760 | 26.3 |
| 2012 | 335,603 | 27.8 | 339,235 | 27.4 | 3,395.0 | 305.0 | 1,149,924,878 | 24.7 |
| 2013 | 342,684 | 26.7 | 335,937 | 26.4 | 3,148.8 | 119.9 | 1,051,785,283 | 24.6 |
| 2014 | 338,623 | 26.4 | 334,928 | 26.7 | 3,274.8 | 134.4 | 1,095,801,468 | 24.0 |
| 2015 | 369,240 | 27.4 | 355,391 | 26.3 | 3,662.0 | 332.6 | 1,282,196,262 | 24.2 |
| 2016 | 365,022 | 27.2 | 370,935 | 27.7 | 4,071.8 | 433.6 | 1,494,055,348 | 26.2 |
| 2017 | 440,432 | 28.1 | 390,265 | 28.3 | 4,007.6 | 437.7 | 1,574,491,214 | 26.4 |

*Source: Original*

In the Southeastern Forest Area, the volume of felling, in the analyzed period, was in the interval 301,703-440,432 $m^3$, where the placement followed the felling dynamics. The highest average price was formed in 2016, influenced by the sale of more valuable assortments, while the most significant gross income was achieved in 2017 (Table 1).

In general, the availability and supply of technical wood affects the supply and demand of the wood industry. This can be explained by the fact that an increased supply of technical wood results in its lower price, and thus lower costs, better competitiveness, and higher demand for wood industry products (Trømborg *et al*., 2000). In those circumstances, the forestry sector is undergoing a transition towards a "circular bioeconomy" (Michal *et al.*, 2021), and the complex interaction between economic and environmental forces is common in most European countries (Lombardo, 2024).

Graph 1. Trends in total logging, total sales, average price, and total income




*Source: Original*

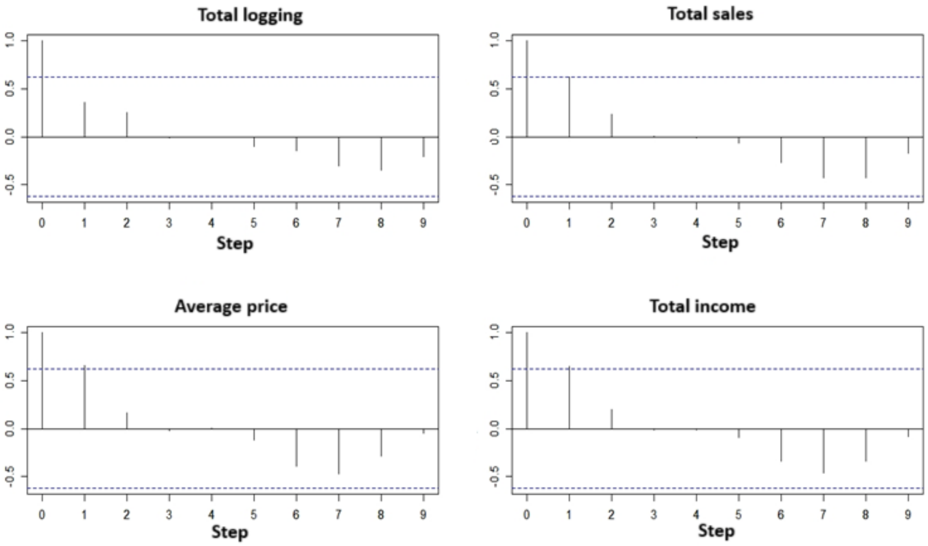
Graph 1 shows total logging (in thousands of cubic meters), total sales (in thousands of cubic meters), the average price of wood (in thousands of dinars), and total gross revenue (in millions of dinars) in the Southeastern Forest Area during the observed period.

In all analyzed cases, shown in Graph 1, an increasing trend can be observed in the categories: total logging, total sales, average price, and total revenue). This indicates the absence of extreme deviations from the trend line, except for 2017, when more intensive logging occurred.

Unlike the formation of prices based on market power and the strength of market participants (Brkić *et al.*, 2017), the realization of wood assortments is carried out according to the current price list of the PE for the current year. This is done according to the sales model on the forest-truck road, where "the wood is cut to a certain length, classified and stacked on the forest road awaiting shipment" (Delić, 2011). This method of sales is "...a variant of free sales, when forest enterprises determine in advance, based on production costs and the market situation, the prices of forest assortments for a certain period, after which they must be revised" (Ranković, Keča, 2011).

In practice, prices are formed under market conditions, primarily the supply and demand ratio and the costs that arise from the moment of felling and processing of trees to the point of delivery (Keča *et al.*, 2015).

Graph 2. Autocorrelation coefficients




*Source: Original*

Graph 2 presents the autocorrelation coefficients of total felling, sales and gross revenue, and the average price in the Southeastern Forest Area. The autocorrelation coefficient with step 1 is significant for total sales, average price, and total revenue. In these cases, the modified Mann-Kendall test was applied.

Table 2. Mann-Kendall test

| **SOUTHEASTERN FOREST AREA** | | | | |
|---|---|---|---|---|
| **Variable** | **Test** | **Statistics** | ***p*-value** | **Trend** |
| Total logging | Mann-Kendall | 3.041 | 0.002 | Yes |
| Total sales | Modif. Mann-Kendall | 3.041 | 0.002 | Yes |
| Average price | Modif. Mann-Kendall | 2.683 | 0.007 | Yes |
| Total gross income | Modif. Mann-Kendall | 3.220 | 0.001 | Yes |

*Source: Original*

Based on the results (Table 2), it was determined that there is a statistically significant increasing trend in total felling, sales, average price, and total revenue in the Southeastern Forest Area in the period 2008-2017.

For the reason of comparison with other forest areas, Table 3 shows the sales and revenue shares for beech, oak, and poplar in 2017. For beech, the percentage of sales and gross income was calculated in relation to the total sales and the income from beech in all forest estates (Marčeta, 2023).

Table 3. Sales and gross income from beech, oak, and poplar in 5 forest areas in 2017

| | **FOREST AREA** | | | | |
|---|---|---|---|---|---|
| | **Central** (%) | **Eastern** (%) | **Western** (%) | **Southwestern** (%) | **Southeastern** (%) |
| **SALES** | | | | | |
| **1.Beech** *Fagus* | 2.47 | 28.68 | 10.40 | 24.55 | **31.67** |
| **2.Oak** *Quercus* | | | | | |
| **Turkey oak** *Quercus cerris* | 3.70 | 2.54 | 2.42 | **16.76** | 11.35 |
| **English oak** *Quercus robur* | **1.13** | - | 0.16 | - | - |
| **Sessile oak** *Quercus petraea* | 2.71 | 4.03 | 6.99 | 12.28 | **13.68** |
| **Italian oak** *Quercus frainetto* | 0.30 | 0.41 | 0.29 | 2.02 | **9.13** |
| **3.Poplar** *Populus* | **62.24** | 19.20 | 3.55 | 0.01 | 0.09 |
| **INCOME** | | | | | |
| **1.Beech** *Fagus* | 2.50 | 27.71 | 10.98 | 25.87 | **31.92** |
| **2. Oak** *Quercus* | | | | | |
| **Turkey oak** *Quercus cerris* | 4.07 | 2.54 | 2.47 | **14.62** | 10.70 |
| **English oak** *Quercus robur* | **3.04** | - | 0.39 | - | - |
| **Sessile oak** *Quercus petraea* | 3.24 | 6.79 | 6.32 | 10.63 | **11.61** |
| **Italian oak** *Quercus frainetto* | 0.23 | 0.33 | 0.35 | 1.51 | **9.01** |
| **3. Poplar** *Populus* | **67.71** | 22.70 | 3.54 | 0 | 0.05 |

*Source: Original*

The results show that the share of beech sales is the highest in the Southeastern Forest Area (31.67%). This is expected, given the dominance of beech in the forest fund of this region. At the republic level, beech makes up about 30% of the forest fund of Serbia, while in state forests it occupies 31%

(2008). In a slightly smaller percentage, beech is represented in sales in Eastern and Southeastern Serbia. In addition, the Southeastern Forest Area has the largest share in the sale of Sessile oak and Italian oak, 13.68% and 9.13%, respectively, while the sale of Turkey oak has the largest share in the Southwest Forest Area (Table 3).

## CONCLUSIONS

Based on the research results, the following conclusions can be highlighted:

-in the period 2008-2017, a constant increase in logging and wood sales was recorded in the Southeastern Forest Area;

-average wood prices increased, with a sharp increase in prices in 2016 when more valuable wood assortments were sold;

-Sessile oak and Italian oak represent a significant share of oak sales and are most represented in the Southeastern Forest Area;

-seasonal fluctuations in wood prices and supply were determined, depending on demand and production in individual years;

-the highest gross revenues were achieved in 2017, which is the result of a larger sales volume and increased average price;

-beech had the largest share in sales in the Southeastern Forest Area, which is in line with its dominance in the forest fund;

-seasonal changes in the price and supply of wood were observed, which depended on market trends and production;

-the results of the Mann-Kendall test confirmed the existence of a statistically significant increase in the volume of felling, sales, prices, and gross income;

-the important role of the PE "Srbijašume" in the context of defining the criteria for the allocation of raw materials is emphasized, thereby affecting the balance between supply and demand on the local market.

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# ALLOZYMATIC DIVERSITY IN A NATURAL POPULATION OF *PINUS HARTWEGII* LINDL. IN VERACRUZ, MEXICO

## SUMMARY

Understanding the degrees and geographic distributions of genetic variation between tree species is important to planning their management and conservation. The genetic variation of the population of *Pinus hartwegii* Lindl., from the National Park "Cofre de Perote" in Veracruz, Mexico, was assessed at two sites of different altitudes (3,500 and 4,000 m.a.s.l.) to aid in the development of a conservation program for this important forest resource. Ten trees were randomly selected at each site, and at least 20 cones were collected per tree. 25 genetic markers were detected from germinated seeds. The average number of alleles per locus was 1.56, with most alleles found at both altitude sites. The expected heterozygosity along the altitude gradient ranged from 0.235 to 0.260. Based on this data, it is recommended to establish a Forest Genetic Resource Conservation Unit (FGRCU) at 4,000 m with a minimum viable population size (Ne) of 7,645 individuals, and another at 3,500 m with a Ne of 8,563 trees.

**Keywords**: Allozyme; Alleles; Altitudinal gradient; Polymorphism

## INTRODUCTION

*Pinus hartwegii* Lindl. Is a species confined to the highest mountains of Mexico and Central America, located between 3000 and 4000 m.a.s.l. Its natural distribution is discontinuous in high mountain sites from Nuevo León in northwestern Mexico (approx. 25° LN) to sites near the border between Guatemala and El Salvador (approx. 14° LN) (Perry, 1991). The importance of *P. hartwegii* is not only focused on the production of wood or wood products, it also great ecological importance because it is one of the most tolerant species to low temperatures in Mexico (Viveros-Viveros *et al*. 2007), fulfilling protective functions for other associated resources and providing services such as carbon sequestration, water retention, and buffering the effects of pollution (Musálem

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and Solís 2000). This species constitutes the altitudinal limit of the arboreal vegetation of the high mountains and volcanoes located in important national parks in Mexico, such as the Colima Volcano, Pico de Tancítaro, Nevado de Toluca, Ajusco, Popocatépetl, Iztaccihuatl, and Malinche (Vera-Vilchis and Rodríguez-Trejo 2007). In Veracruz, Mexico, it is found in fragmented populations on the Nauhcampatépetl (Cofre de Perote) and Citlaltépetl (Pico de Orizaba) (Iglesias *et al.* 2012).

The unique and significant elevation distribution of *P. hartwegii* makes it highly susceptible to global warming. In Mexico, it is projected that the average annual temperature will increase by 1.5°C, 2.3°C, and 3.7°C by 2030, 2060, and 2090, respectively, accompanied by a decrease in annual precipitation by 6.7%, 9.0%, and 18.2%, respectively (Sáenz-Romero *et al.* 2010). These changes are expected to lead to a reduction in the habitat of *P. hartwegii* by up to 42% (Arriaga and Gómez 2004). Research by Alfaro-Ramírez *et al*. (2020) suggests a potential loss of distribution area ranging from 29 to 70% in the next 50 years, while García-Amorena *et al.* (2021) anticipate a reduction ranging from 6 to 23% by 2050 and from 6 to 44% by 2070. Furthermore, the recent increase in temperature has resulted in a 10.6% reduction in the species' relative growth (Ricker *et al*. 2007).

The analysis of cones and seeds is a useful tool for understanding the reproductive status of conifers (Owens *et al*. 2005). This is because reproductive characteristics are important for understanding genetic processes in a population and for monitoring the viability of populations (Rajora *et al*. 2000). Due to the low viability of seeds and the high proportion of empty seeds, the reproduction rate of the "Cofre de Perote" population has experienced a reduction (Iglesias-Andreu *et al*. 2006). Given this, it is crucial to know the distribution of the species as well as the patterns of genetic variation within its populations. This species is better adapted to elevations of 3800 m.a.s.l. on Mount Tlaloc and 3900 m.a.s.l. on Nevado de Toluca, based on reproductive indicators. Both populations have shown comparable levels of inbreeding depression associated with seed production across altitudes. These reproductive indicators were affected by environmental factors in the Monte Tláloc and Nevado de Toluca populations, as well as in each of the *P. hartwegii* populations at different altitudes (Andrade-Gómez *et al*. 2021).

Variation in allozyme composition has been widely used since 1960 to investigate patterns of genetic variation in conifers due to its simple Mendelian inheritance and co-dominant expression (Hamrick and Godt 1996). The use of haploid (n) tissue from the megagametophyte of conifer seeds has been used to assess genetics and facilitate genetic studies. Through the use of allozyme, genetic variation has been studied in several conifer species, e.g. in *Pinus cembra* L. and *P. sibirica* Du Tour (Politov *et al*. 2008), *Pinus sylvestris* L. (Bilgen and Kaya 2007; Przybylski *et al.* 2020), and *Pinus nigra* J.F. Arnold (Scaltsoyiannes *et al*. 2009). Knowledge of the genetic variation in species or populations allows the establishment of better management or conservation strategies (Przybylski *et al.* 2020).

Although this species is important, especially in the *P. hartwegii* population of the "Cofre de Perote" in Mexico, little is known about the effects of altitude on the genetic variation levels of this species. Moderate genetic differentiation was found along the altitudinal gradient in the *P. hartwegii* population from Michoacán State in the “Pico de Tancítaro” National Park of Mexico, according to a study conducted there. For this reason, this study was performed to evaluate the genetic variation of the *P. hartwegii* population at "Cofre de Perote" in two locations along an altitudinal gradient, to devise an efficient management and conservation strategy.

## MATERIAL AND METHODS

The *P. hartwegii* population of the “Cofre de Perote” National Park in Veracruz, Mexico, is located at 19°15' N latitude and 97º W longitude, in the central-western part of the Veracruz state (Fig. 1). In this rugged and mountainous area, two sites were located along an altitudinal gradient, one at 4000 m.a.s.l. (Location 1) and the other at 3,500 m.a.s.l (Location 2).

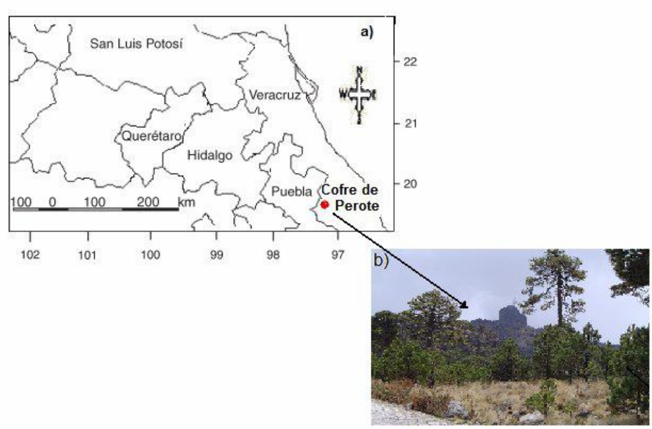


Fig.1. a) Location of *P. hartwegii* population in “Cofre de Perote”, Veracruz, Mexico. b) *P. hartwegii* forest.

The geographic area under study is characterized by rocky but moderately deep soils, poor organic matter composition, and an average annual temperature of 12 °C (SMN, 1984). Bulk seed lots were used for the population study; each seed lot was collected from 10 trees per altitudinal site. In 2008, at least 20 cones were gathered from each tree's central crown. At room temperature, the seeds were germinated in Petri dishes filled with moist agrolite. The megagametophyte of each seed was removed for enzymatic examination when the roots were three to five millimeters long.

200 µL of 0.2 M phosphate extraction buffer, pH 7.5, was used to cold homogenize the samples (Hodgkiss, 1998). After centrifuging the samples for ten minutes at 14,000 rpm, the supernatants were subjected to electrophoresis. Isoenzymes in polyacrylamide gels were examined using a discontinuous vertical electrophoresis system (Ornstein, 1964).

For the electrophoretic separations, separation and compaction polyacrylamide gels at 12.5% and 5%, respectively, and Tris-Glycine buffer pH 8.3, were used. These were carried out at 160 volts for 5 hours. The staining of nine isoenzyme systems followed conventional protocols (Vallejos, 1983) with slight modifications. Still, only three of them (Esterase, EST: E.C. 3.1.1.2, aspartate aminotransferase, GOT: E.C. 2.6.1.1, and Acid phosphatase, ACP: E.C. 3.1.3.2) showed good resolution and repeatability. Different loci encoding the same allozyme were designated based on their relative mobility. Intraspecific enzyme mobility was verified by comparing the different isomorphs in the same gel.

The data obtained from electrophoretic variation at different altitudinal sites was used to establish a matrix. Various population genetic parameters such as allele richness (A), percentage of polymorphic loci with a 99% polymorphism criterion, and expected heterozygosity (He) were calculated for each altitude level using TFPGA software (Miller, 1997). A cluster analysis was conducted based on genetic distances between the study sites using the Nei method (Nei, 1972) and the unweighted pair group method with arithmetic means (UPGMA). The effective size of the viable population was determined using the expected heterozygosity value and a regression formula based on a mutation rate of 1×10-5, as per data from Millar and Libby (1991) for coniferous species, and the equation of Crow and Kimura (1972). The regression formula is Ne=-984.58+ (36723 He).

## RESULTS AND DISCUSSION

Twenty-five loci were identified for the three isozyme systems analyzed. Esterase isoenzymes had a greater number of polymorphic loci (Table 1).

Table 1. Allozyme composition in a *P. hartwegii* population from “Cofre de Perote”, Veracruz, Mexico

| **Isoenzyme System** | ***Loci*** | | |
|---|---|---|---|
| | **Polymorphic** | **Monomorphic** | **Total** |
| ACP | 2 | 1 | 3 |
| EST | 12 | 0 | 12 |
| GOT | 9 | 1 | 10 |
| Total | 22 | 3 | 25 |

*ACP: Acid Phosphatase (EC. 3.1.3.2); EST: Esterase (EC. 3.1.1.1); GOT: aspartate aminotransferase (EC. 2.6.1.1)*

A substantial isoenzyme variation was found between the altitudinal sites evaluated. The altitudinal site of 4,000 m.a.s.l. showed the presence of a smaller number of polymorphic loci, while the altitudinal site of 3,500 m.a.s.l. was the one that showed the greatest polymorphism. This could be due to a differential genetic variation along the altitudinal transect examined (Table 2).

High values of genetic diversity were observed because of relatively high values of the number of polymorphic loci, the mean number of alleles per locus, and the heterozygosity observed in both sites. However, some differences were observed between them (Table 2).

Table 2. Isoenzyme variation and genetic diversity estimation of two altitudinal locations from “Cofre de Perote”, Veracruz, Mexico

| Locations | Isoenzyme systems | | | | | | Percentage *Loci* Polymorphic (P) | Expected heterocigocity (He) |
|---|---|---|---|---|---|---|---|---|
| | ACP | | EST | | GOT | | | |
| | P | M | P | M | P | M | | |
| Location 1 | 2 | 1 | 9 | 3 | 4 | 6 | 56 | 0.235 |
| Location 2 | 1 | 2 | 10 | 2 | 6 | 4 | 68 | 0.260 |
| Population | - | - | - | - | - | - | 62 | 0.250 |

*ACP: Acid Phosphatase (EC. 3.1.3.2); EST: Esterase (EC. 3.1.1.1); GOT: aspartate aminotransferase (EC. 2.6.1.1); P: Polymorphic loci; M: Monomorphic loci: Location 1: 4000 m.a.s.l; Location 2: 3500 m.a.s.l.*

The average number of alleles per locus (A) was 1.56. Some alleles varied in frequency, but it was found that both sites shared 56% of the allele variants detected (Fig. 2).

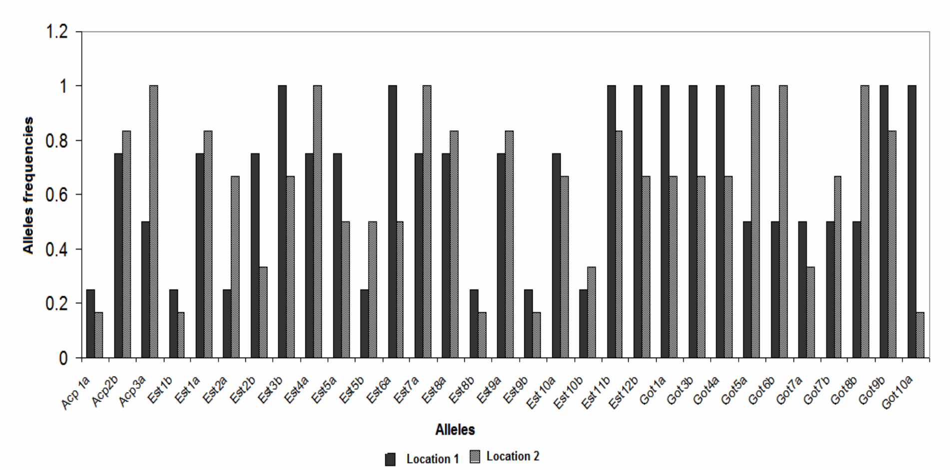


Fig. 2. Allele frequency variation for loci showed in two altitudinal locations of *P. harwegii* from “Cofre de Perote”, Veracruz, Mexico

However, 44% of the alleles detected were specific to one site or another. Based on these results, a Nei (1978) genetic identity of 0.877 and a genetic distance (Nei 1972) of 0.131 were estimated (Fig. 3).

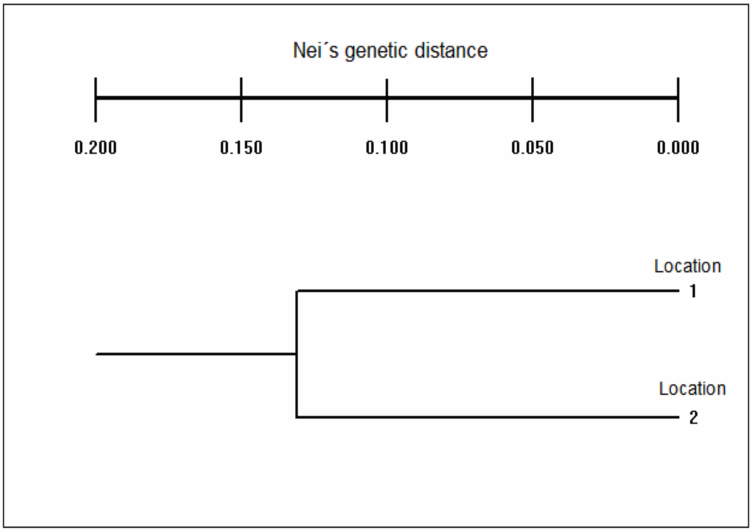


Fig. 3. UPGMA Dendrogram for the two altitudinal locations of *P. hartwegii* based on the Nei method (Nei 1972)

The close genetic distance observed shows that the average difference between these sites does not exceed 1% of the structural genes. The subpopulation located at the highest altitude presented six alleles: $ACP_3$-b, $EST_4$-b, $EST_7$-b, $GOT_5$-b, $GOT_6$-a, and $GOT_8$-a, which varied in frequencies between 0.25 and 0.5. In comparison, the subpopulation located at a lower altitude presented nine alleles: $EST_3$-a, $EST_6$-b, $EST_{11}$-a, $EST_{12}$-a, $GOT_1$-b, $GOT_3$-a, $GOT_4$-b, $GOT_9$-a, and $GOT_{10}$-b, with frequencies that ranged between 0.167 and 0.833. Finally, it was found that the minimum population size (Ne) estimated for the lowest altitude site was 8,563 individuals, while for the highest altitude site, it was 7,645 individuals.

Conifers constitute one of the groups of species with the greatest genetic variability (Ledig, 1998), and the population of *P. hartwegii* from “Cofre de Perote”, Veracruz, is no exception. The average number of alleles per locus (A) detected in this population was like that found when analyzing nuclear DNA, mitochondrial DNA, and chloroplast DNA (1.7, 1.5, and 1.6, respectively) of this species in the protection area of flora and fauna of “Nevado de Toluca” (Heredia-Bobadilla *et al*. 2019).

The average value of polymorphic loci (62%) detected in this population was higher than that found in “Pico de Tancítaro”, located in Michoacán, Mexico (58.3%) (Viveros-Viveros *et al*., 2010) and that of mitochondrial DNA (33.3%) detected in the “Nevado de Toluca” flora and fauna protection area (Heredia-Bobadilla *et al*., 2019). This value can be considered high, like that detected in other species of the genus, such as *Pinus nigra* (74%; Turna *et al*. 2006), *P. sylvestris* (76.5%; Korshikov *et al*. 2005), and *P. hartwegii*, in which its variation in nuclear DNA was evaluated (78.9%) (Heredia-Bobadilla *et al.* 2019).

The expected heterozygosity estimates of 0.25 could be considered high. Few species belonging to the *Pinus* genus have demonstrated expected heterozygosity values exceeding 0.20. Hiebert and Hamrick (1983) estimated values between 0.189 and 0.227 for the expected heterozygosity of 41 *Pinus* species. Some species have values higher than 0.340, such as *P. longaeva* D. K. Bailey. The variation values identified in this study agree with the findings of Delgado *et al.* (1999), who have demonstrated that conifer populations in Mexico and Central America exhibit higher levels of polymorphism and differentiation than other populations situated at higher latitudes.

It has been suggested that the genetic variation in *Pinus* spp., is a consequence of relatively high rates of cross-pollination (Hamrick and Godt, 1989; Hamrick *et al.* 1992) and the effective gene flow associated with long-distance pollen dispersal or homogeneity selection pressure (Wheeler and Guries 1982). At the site located at 3,500 m.a.s.l. greater variation was found, with greater allele richness: 42 total alleles (nine specific), a higher level of polymorphism (68%), greater heterozygosity (0.26), and a lower number of fixed alleles (8). While at the site, located at 4000 m.a.s.l. less variation was found, with 39 total alleles (six specific) and 56% polymorphism, as well as a heterozygosity of 0.235 and a greater number (11) of fixed alleles.

The variations in allele composition detected between sites could be an adaptive response of the population to spatial heterogeneity (Furnier and Adams 1986). Based on the specific and fixed alleles found, some ecological elements that are primarily related to topography and altitude may constitute the structural elements of an allele pattern. Another possibility is that bottlenecks that arise during range expansion cause genetic variation to decline during forest movements (Newton *et al.* 1999). In forest species, Ohsawa and Ide (2008) have described similar cases in which populations at higher altitudes have comparatively less variation.

It is interesting to note that this population, like others of the same species, faces problems of fragmentation and isolation. The results obtained in this work indicate that it is convenient to apply permissive sampling strategies to preserve the allele richness existing at different altitudinal levels. Conservation programs should include both, representative genes and specific genes, to preserve maximum allele richness. These results provide information for the first time on the genetic variability of *P. hartwegii* from the “Cofre de Perote”, despite the relatively small number of loci and altitudinal gradients considered in this study. To conserve a representative sample of current genetic diversity, it is recommended that at least one forest genetic resources' conservation unit (FGRCU) be established at 4,000 m with a minimum viable population size (Ne) of 7,645 individuals, and another at 3,500 m with a Ne of 8,563 trees.

However, if the current global warming trend continues (Sáenz-Romero *et al*. 2010), an assisted migration to higher altitude areas in other mountains would be necessary. Seeds would have to be collected from current populations, the plants reproduced in a nursery, and new ex-situ conservation plantations established at a site about 400 m above sea level higher than the seed collection site. This would allow the populations to readjust to the climate to which they are adapted. According to estimates for this species based on the Canadian General Circulation Model and the A2 model emission scenario (Sáenz-Romero *et al.* 2010), the climate in 2030 will be about 400 m higher than today. Higher elevation populations such as the “Cofre de Perote” would no longer have a place to migrate, as its peak is 4,282 m above sea level. Therefore, migration to other high mountains or volcanoes of the Mexican volcanic axis, such as “Iztaccihuatl, Popocatepetl, or Citlaltepetl” (Pico de Orizaba), should be encouraged. It can be argued that local populations of *P. hartwegii* on these volcanoes may be suffering from inbreeding depression due to gene flow from foreign plantations. However, it should be considered that local populations would also progressively lose their adaptation to climate change, which would undoubtedly represent a greater risk in the long term. It is hoped that future studies that include a greater number of molecular and population markers will provide a better understanding of the levels and patterns of the genetic structure of this important and valuable forest resource.

## CONCLUSIONS

The population of *P. hartwegii* in the “Cofre de Perote” in Veracruz, Mexico, was found to have a high level of genetic diversity. The lower altitude site has higher genetic diversity and allelic richness than the higher altitude site, due to the pattern of distribution of genetic diversity observed. Based on this data, it is advisable to establish a Forest Genetic Resource Conservation Unit (FGRCU) at altitudes of 3,500 m and 4,000 m.

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# THE INFLUENCE OF SOIL TYPE AND CLIMATIC CONDITIONS ON THE YIELD AND MORPHOLOGICAL CHARACTERISTICS OF POTATOES

## SUMMARY

Soil is the basic substrate, which, with its physical, chemical and biological properties, greatly influences the productivity of cultivated plants. Soil investigations are often reduced to a minimum, and one of the goals of this research is to give importance to the land itself and its influence on both the yield and the quality of cultivated plants.

Research in the open field was carried out in a two-year period (2017-2018) at three locations with different altitudes (550, 90 and 1100 m) and three different types of soil (Eastern Sarajevo - fluvisol soil type, Rogatica - district cambisol soil type, Bijeljina - soil type humofluvisol).

The field research included factors such as soil type (A) and year (B).

The tested soil types differed significantly in terms of chemical, physical and microbiological characteristics and, in combination with climatic factors, significantly influenced the yield and morphological characteristics of potatoes. The yield of potatoes in the open field was significantly influenced by the type of soil and the year. Potatoes grown in the Bijeljina location (humofluvisol) had the highest yields, and the lowest in the Istočno Sarajevo location (fluvisol). Higher yields were achieved in 2018, compared to 2017, which was mostly influenced by the agroecological conditions at the time of the test. The favorable physical, chemical and microbiological properties of the humofluvisol soil in the Bijeljina locality influenced the yield of potato tubers, so the highest yield was achieved in this locality.

**Keywords**: soil type, climate conditions, tuber yield, morphological characteristics of potatoes

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## INTRODUCTION

Potato is a nutritiousvegetable that is regarded as one of the most beneficial crops for reducing hunger, malnutrition and poverty around the world because of its great productivity, as can be seen by its remarkably high harvesting index of over 75% (Wijesinha-Bettoni and Mouille B., 2019). Hence, potato has the potential to significantly contribute to human nutrition as an addition to other staple crops like rice and wheat (Campos and Ortiz, 2020). For food security, understanding the effects of climate change on potato yield is critical. Extreme heat and drought brought on by global climate change pose a significant threat to long-term crop production by reducing plant performance and output. Such a negative influence on agicultural productivity is projected to worsen in the future (Chourasia *et al.*, 2021) due to increasing greenhouse gas emissions which will lead to increased evapotranspiration and drought severity (Dahal *et al*., 2019).

The growth and quality of potatoes are influenced by environmental factors such as temperature, humidity, light, soil type and nutrient content (Khan *et al*., 2011). Potato is a plant with specific and moderate temperature requirements according to many authors. A combined effect of temperature and precipitation is crucial (Borkowska and Grundas, 2007). High temperature can decrease yield due to physiological and biochemical changes occurring in the plant, suchas photosynthesis, respiration and water status. A negative impact of too high temperature can, however, be partially reduced by evenly distributed optimum precipitation. Unlike most other plant species for which the soil serves only as a substrate from which they take water and nutrients through the root system, potatoes also form vegetative organs in the soil - tubers, and form other vegetative organs with fruit (berries) above the surface of the soil. As much as 80% of the total mass of the potato is formed in the soil (Lazić *et al*., 1998). Potatoes have great requirements regarding the air regime of the soil. The soil must be sufficiently loose. On compacted soil, the stolons branch and form small tubers, and therefore it is preferable to grow potatoes on soils with a light mechanical composition. As a result of soil compaction, the soil structure deteriorates, which determines the total and differential porosity, and in this connection the water-air, thermal and biological regime of the soil deteriorates. Deterioration of these land regimes adversely affects obtaining high and stable yields of appropriate quality (Nikolić *et al.*, 2003). The aim of this study was to determine the influence of climatic and soil factors on the morphological characteristics and yield parameters of potatoes. Different types of tested soil in combination with variable climatic factors (precipitation and air temperature) will affect the variation of potato yield, as well as the resulting tubers will have different quality and market value.

Productive properties of potatoes are significantly influenced by non-genetic factors, i.e. factors of the external environment, such as soil and climate factors.

## MATERIAL AND METHODS

Field experiments were conducted in area Bosna and Herzegovina (Fig.1) in three different localities, which significantly differed from each other in terms of soil types, fertility, altitude (550, 90, 1100 m), and climatic conditions. The research was carried out over two years (2017, 2018) at three locations: in the municipality of Istočna Ilidža - experimental field of the Faculty of Agriculture, soil type fluvisol (Fig.4); in Bijeljina (Fig.2) - on the private estate of the Perković family in the village of Kojčinovac, soil type humofluvisol; and in Rogatica (Fig. 3) - on the property of Solanum produkt in Borike, soil type district cambisol. The medium-to-late Agria potato variety was used as plant material for planting potatoes. Table 1. shows the factors used in this research.

Table 1. Factors of research in the field

| No. | Factors | Treatment |
|---|---|---|
| 1. | Soil type (A) | $A_1$ – fluvisol,<br>$A_2$ - humofluvisol,<br>$A_3$ – district kambisol |
| 2. | Year (B) | $B_1$ – first year,<br>$B_2$ – second year |

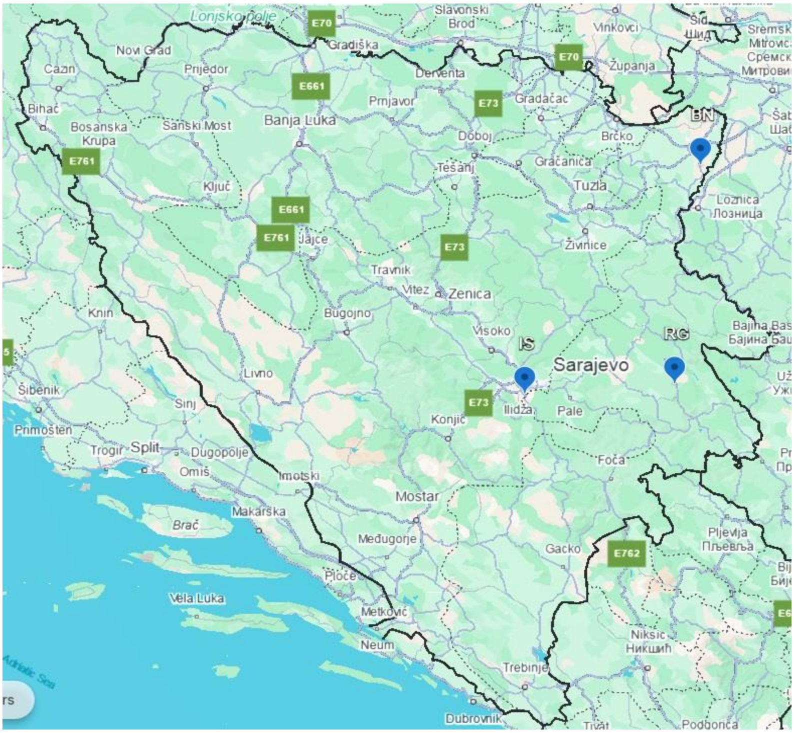


Figure 1. Location of field experiment

Meteorological data on average monthly temperatures and precipitation were taken from the Hydrometeorological Institute of the Republic of Srpska and the Federal Hydrometeorological Institute of Bosnia and Herzegovina, from the measuring stations closest to the locations of the field experiments. From the morphological and productive characteristics of potatoes in the full flowering phase, the following were determined: plant height (cm) (measured from the surface of the land to the top of the upper flower); the number of above-ground shoots and the fresh above-ground mass of the plant (g).

At the end of the vegetation period, the following were analyzed: the number of tubers per house; average tuber mass (g); the yield of potato tubers, which was calculated per hectare (t $ha^{-1}$) and tuber fraction structure. The number of above-ground shoots and their height (cm) were determined on a sample of twenty houses from each basic plot, and the fresh mass of the plant was determined on a sample of 5 houses. To determine the number of tubers per house, the average weight of the tuber (g), the yield of tubers and the fractional structure of the tubers, samples of twenty houses were taken from each basic plot at the technological maturity of potatoes.

The data obtained from the two-year experiments in the field (AxB) were processed using the method of descriptive statistics. The significance of the differences between the treatments was tested by analysis of variance (ANOVA), and the correlation of the phenomena by correlation-regression analysis. The significance of differences was tested by Fisher's LSD test. Statistical processing of the obtained data was done using the statistical program STATISTICA 10 (StatSoft, Inc. Corporation, Tulsa, OK, USA).

Figure 2. Locality Kojčinovac - Bijeljina (soil type humofluvisol)

Figure 3. Locality Borike - Rogatica (soil type district kambisol)

Figure 4. Locality Kula – Istočno Sarajevo (soil type fluvisol)

## RESULTS AND DISCUSSION

Air temperature and precipitation are the meteorological elements that have the greatest influence on the volume of plant production, the height and quality of the yield of cultivated plants. The average annual temperatures in East Sarajevo and its surroundings in 2017 were 11 °C, and in 2018 they were 11.4 °C and were higher than the multi-year average (Table 2), and during the potato growing season, the average monthly temperatures in 2017 in 2018 (17.5 °C) and in 2018

(17.7 °C) were higher than the long-term average (16.2 °C). In 2017, the average monthly temperatures for April, May and September were lower compared to the average temperatures for these months in 2018. The total amount of precipitation for East Sarajevo and its surroundings in 2017 was 937.3 mm, and in 2018 it was 1043.3 mm, which is more than the multi-year average (932 mm). The average annual temperature for Bijeljina and its surroundings in 2017 was 12.9 °C, and in 2018 it was 13.3 °C and were higher than the long-term average of 11.5 °C (Table 2), while ongoing potato vegetation mean monthly temperatures in 2017 (19.83 °C) and 2018 (20.58 °C) were higher than the multi-year average (18.18 °C). In 2017, average monthly temperatures were lower in April, May and September than average temperatures for the same period in 2018. The total amount of precipitation for Bijeljina and its surroundings in 2017 was 678.9 mm, and in 2018 it was 717.6 mm, which is lower than the multi-year average (778.2 mm). The average annual temperature for Rogatica (Borike) in 2017 was 7.9 °C, and in 2018 it was 8.6 °C and were higher than the long-term average of 7.0 °C (Table 2), while potato vegetation mean monthly temperatures in 2017 (16.1 °C) and 2018 (15.66 °C) were higher than the multi-year average (14.46 °C). In 2017, during the potato growing season, the average monthly temperatures were lower than the average temperatures for the same period in 2018 for May and September. The total amount of precipitation in 2017 was 949.9 mm, which is less compared to 2018, in which a total of 974.2 mm of precipitation was recorded and more than the multi-year average (859.0 mm).

Table 2. Average monthly air temperatures (°C) and precipitation (mm) in 2017 and 2018 and multi-year average

| Year \ Month | | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | Average / sum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. Sarajevo | 2017. | °C | -4,8 | 5,2 | 8,5 | 9,2 | 15,3 | 20,3 | 21,8 | 22,6 | 15,5 | 10,4 | 5,4 | 2,2 | 11,0 |
| | | mm | 57,9 | 69,1 | 43.6 | 132,4 | 73,8 | 55 | 66,5 | 38,7 | 93,2 | 89,3 | 74,9 | 142,9 | 937,3 |
| | 2018. | °C | 4,1 | 0,6 | 5,4 | 14,8 | 16,6 | 17,9 | 19,7 | 20,7 | 16,2 | 13,1 | 7,6 | 0,5 | 11,4 |
| | | mm | 66,7 | 76,7 | 109,8 | 61,4 | 178,1 | 131,5 | 119,6 | 87,5 | 25,9 | 47,5 | 54,7 | 83,9 | 1043,3 |
| | 1981-2010 | °C | -0,1 | 1,4 | 5,3 | 9,9 | 15,0 | 17,7 | 19,8 | 19,7 | 15,1 | 10,9 | 5,2 | 1,1 | 10,1 |
| | | mm | 67 | 63 | 71 | 78 | 73 | 94 | 72 | 70 | 86 | 85 | 91 | 85 | 932 |
| Bijeljina | 2017. | °C | -4,9 | 5,2 | 10,6 | 11,6 | 17,9 | 23,2 | 24,6 | 24,7 | 17,0 | 12,6 | 7,4 | 4,7 | 12,9 |
| | | mm | 35,3 | 45,1 | 42,4 | 92,8 | 67,5 | 39,9 | 47,3 | 35,4 | 102,3 | 60,9 | 45,7 | 64,3 | 678,9 |
| | 2018. | °C | 4,7 | 1,4 | 5,6 | 16,8 | 20,0 | 21,4 | 22,8 | 24,1 | 18,4 | 14,5 | 7,9 | 2,5 | 13,3 |
| | | mm | 65,4 | 82,3 | 88,6 | 34,6 | 91,7 | 125,4 | 53,5 | 16,0 | 29,9 | 27,4 | 35,4 | 67,4 | 717,6 |
| | 1981-2010 | °C | 0,3 | 1,9 | 6,9 | 11,8 | 16,9 | 19,9 | 22,1 | 21,2 | 17,2 | 11,6 | 6,0 | 2,3 | 11,5 |
| | | mm | 54,1 | 43,5 | 59,7 | 66,1 | 68,2 | 100,0 | 74,6 | 60,7 | 55,8 | 67,6 | 64,0 | 63,9 | 778,2 |
| Borike | 2017. | °C | -8,0 | 2,1 | 5,2 | 6,0 | 12,7 | 17,5 | 18,6 | 19,2 | 12,5 | 7,4 | 2,2 | -0,7 | 7,9 |
| | | mm | 50,6 | 51,2 | 43,8 | 165,1 | 51,7 | 90,0 | 97,8 | 16,6 | 85,8 | 121,7 | 67,9 | 107,7 | 949,9 |
| | 2018. | °C | -0,1 | -1,8 | 2,9 | 11,6 | 14,3 | 15,5 | 17,6 | 18,1 | 12,8 | 9,4 | 4,5 | -2 | 8,6 |
| | | mm | 60,5 | 65,5 | 85,1 | 49,1 | 89,5 | 166,5 | 136,7 | 117,2 | 43,1 | 27,6 | 60,0 | 73,4 | 974,2 |
| | 1981-2010 | °C | -3,5 | -1,9 | 2,4 | 6,9 | 11,8 | 14,7 | 17,0 | 16,3 | 12,5 | 7,7 | 1,9 | -2,0 | 7,0 |
| | | mm | 52,7 | 52,9 | 56,8 | 65,3 | 73,9 | 93,7 | 74,9 | 72,1 | 80,2 | 78,0 | 88,6 | 69,9 | 859,0 |

Morphological characteristics of potatoes such as: plant height, number of trees in a potato bush, plant weight directly affect the productive characteristics, total yield and quality of potatoes. Table 3 shows the morphological characteristics of potatoes tested in 2017 and 2018 at selected localities (Rogatica, East Sarajevo and Bijeljina) on different types of land.

Table 3. Morphological characteristics of potatoes from the study of localities and types of land in the years of testing

| | | Plant height (cm) | Number of trees in a potato bush | Plant weight (g) |
|---|---|---|---|---|
| Soil type | fluvisol ($A_1$) | 59,2b | 3,0 | 230,0a |
| | humofluvisol($A_2$) | 54,2b | 3,5 | 212,2a |
| | district kambisol ($A_3$) | 65,4a | 3,2 | 147,5b |
| Year | 2017 ($B_1$) | 53,2b | 2,9 | 207,5 |
| | 2018 ($B_1$) | 66,1a | 3,6 | 185,7 |
| 2017($B_1$) | fluvisol ($A_1$) | 59,2bc | 2,8bc | 238,8 |
| | humofluvisol ($A_2$) | 45,8d | 3,8ab | 236,2 |
| | district kambisol ($A_3$) | 54,5c | 2,3c | 147,5 |
| 2018 ($B_1$) | fluvisol ($A_1$) | 59,2bc | 3,3abc | 221,2 |
| | humofluvisol ($A_2$) | 62,7b | 3,3abc | 188,2 |
| | district kambisol ($A_3$) | 76,2a | 4,3a | 147,5 |
| Soil type | | * | ns | * |
| Year | | ** | ns | ns |
| Soil type *x* Year | | * | * | ns |

*Values marked with different lowercase letters per column for year, locality and their interaction are significantly different at the P≤0.05 level according to the LSD¬test; **F test significant at level; ns¬ F test is not significant.*

The height and growth of potatoes depends on the variety, climatic conditions and applied agrotechnical measures (Singh and Ahmed, 2008). In the two-year tests, the height of the plants was highly influenced by the year, while the soil type and the interaction of locality x soil type had a significant effect. At the location in Rogatica (soil type district cambisol), potatoes had the largest stems, and the smallest in Bijeljina (soil type humofluvisol). In the second year of testing, the height of the stems was higher compared to the first year of testing, and Oljača (2016) obtained similar results in her three-year research, where the influence of the year of research is evident.

The number of trees in a potato bush varies depending on the variety, production conditions, the size of the planted tuber (Khan *et al.*, 2004; Poštić *et al.*, 2012) and affects the number of transplanted tubers (Jovović, 2001; Khan *et al.*, 2004). Bus and Wustman (2007) claim that the optimal number of trees is achieved by planting the optimal number of tubers. The number of trees depends on temperature conditions, and the amount and distribution of precipitation during the intensive formation and growth of above-ground organs. Levy and Veilleux (2007) state similarly.

The number of trees per plant in the two-year surveys and on the three land types was influenced by the land type x year interaction. The largest and smallest number of trees had potatoes grown on the land type district cambisol (Rogatica), the smallest was in the first and the largest in the second year of the test. During the second year of testing, there was no difference between the other two localities and soil type, and in the first year of testing, the highest number of trees per plant was on the soil type humofluvisol (Bijeljina) (Table 3). A significant influence of production conditions and year on the number of trees per plant was determined by Momirović *et al.* (2016). Higher air temperatures affect the higher productivity of above-ground mass (Tadesse *et al*., 2001). The obtained results indicate that the largest mass of potato plants was in the location of East Sarajevo, which had lower temperatures than Bijeljina, while the lowest mass was of plants in the location of Rogatica, which has the lowest temperatures. The obtained results are partially in agreement with (Tadesse *et al*., 2001). The influence of the year and the interaction of soil type x year on the plant weight was not determined.

The number of tubers per house was significantly influenced by the year, while the influence of the interaction type of land x year was highly significant. The number of tubers per plant is a varietal trait, but it also depends on the number of trees per plant, agroecological conditions and technological conditions (Barkley, 2005; Poštić *et al.*, 2012). Meteorological conditions in combination with the land affect the extent to which these characteristics will be manifested. In the first year of testing, there was a greater number of tubers. This year is characterized by lower temperatures compared to the second year of testing. The presented results are in accordance with the results of Barkley (2005), who in his research obtained a higher number of tubers per plant at lower than at higher air temperatures. The largest number of tubers per plant was at the location in East Sarajevo (soil type fluvisol) during the second year of testing, while in the first year of testing the fewest tubers were at the location of Bijeljina (soil type humofluvisol) (Table 4).

The average mass of tubers was significantly influenced by soil type and year, while the influence of the interaction was highly significant. Tubers produced on the humofluvisol soil type (locality Bijeljina) had the highest average mass, and the smallest on the soil type fluvisol (locality East Sarajevo) (Table 4). Average tuber weight is a varietal characteristic, but it also depends on agroecological conditions, agrotechnical measures, number of trees per plant, number of tubers per plant (Poštić *et al*. 2012). Higher temperatures stimulate vegetative development, reduce tuber formation and average tuber weight and tuber yield (Tadesse *et al*., 2001). The lowest average mass of the tuber per plant was determined in the first year of the test when in June, July and August there were higher air temperatures and less rainfall, compared to the second year in which the tubers had a higher average mass, which is in accordance with the results Tadesse *et al.* (2001). Higher air temperatures stimulate the vegetative development of the above-ground assimilative part and delay the initiation of stolons and tubers and the earlier development of tubers (Tadesse *et al*., 2001).

Table 4. Yield components and potato yield in the years of testing on selected land types

| | | The number of tubers per house | Average mass of tubers (g) | Tuber yield (t $ha^{-1}$) |
|---|---|---|---|---|
| Soil type | fluvisol ($A_1$) | 10,9 | 72,7b | 41,2b |
| | humofluvisol ($A_2$) | 9,1 | 104,2a | 48,8a |
| | district kambisol ($A_3$) | 10,5 | 87,8ab | 42,0b |
| Year | 2017 ($B_1$) | 11,2a | 76,3b | 41,3b |
| | 2018 ($B_1$) | 9,2b | 100,2a | 46,7a |
| 2017($B_1$) | fluvisol ($A_1$) | 11,3b | 66,0bc | 39,3 |
| | humofluvisol ($A_2$) | 8,3cd | 112,7a | 47,1 |
| | district kambisol ($A_3$) | 14,0a | 50,1c | 37,4 |
| 2018 ($B_1$) | fluvisol ($A_1$) | 10,5bc | 79,3bc | 43,0 |
| | humofluvisol ($A_2$) | 10bc | 95,7ab | 50,4 |
| | district kambisol ($A_3$) | 7d | 125,5a | 46,5 |
| Soil type | | ns | * | * |
| Year | | * | * | * |
| Soil type *x* Year | | ** | ** | ns |

As a consequence of higher temperatures, the number of tubers per plant, the average mass of tubers and the yield of tubers decrease (Tadesse *et al.,* 2001). Air temperatures close to optimum in the month of June in the stage of stolon formation, tuber seeding and the beginning of tuber filling, which were accompanied by a high amount of precipitation, played a key role in the formation of larger tubers, as proven by the following authors (Jovović, 2011; Poštić *et al.*, 2012). In 2017, tubers produced on district cambisol (Rogatica) had the smallest mass, and tubers produced on humofluvisol (Bijeljina) had the largest mass, while in 2018, tubers produced on fluvisol (Istocno Sarajevo) had the smallest mass, and tubers produced on district cambisol had the largest mass (Rogatica) (Table 4). The yield of potatoes depends on the variety and its genetic potential, agroecological conditions, the level of applied agricultural techniques, the size of the seed tuber, the number of trees per plant and the number of tubers (Poštić *et al.*, 2012). Jovović (2001) states that there is a strong relationship between tuber yield and number of trees per plant, as well as between yield, tuber size and number of trees per plant.

In two-year tests on three types of land, the yield was significantly influenced by the type of land and the year, while the influence of the interaction was not significant. Potatoes grown on humofluvisol soil type (Bijeljina locality) had the highest yields, and the lowest on fluvisol soil type (Istočno Sarajevo locality). Higher yields were achieved in 2018, compared to 2017. These results were influenced by the agroecological conditions at the time of the test, as well as the average mass of the tuber. The distribution of precipitation during the growing season in 2018 fully satisfied the water needs of potatoes, which was reflected in the total yield of tubers being significantly higher compared to the previous year of testing. Such results are in accordance with the research of many authors (Milić *et al.*, 2010; Momirović *et al.*, 2010; Poštić *et al*., 2012), who state that production conditions affect the total yield of potato tubers. The yield variation depends on the type of land, relief, physical and chemical properties of the land and availability of nutrients (Penney *et al*., 1996), which is in accordance with our research. The most favorable physical, chemical and microbiological properties of the soil at the Bijeljina location influenced the yield of potato tubers, so the highest yield was achieved at this location.

## CONCLUSIONS

The agroecological conditions of production in the examined years at the three investigated localities differed significantly, 2018 was significantly more favorable in terms of rainfall compared to 2017 for all three investigated localities (Kula-Istočno Sarajevo, Borike-Rogatica and Kojčinovac-Bijeljina). High temperatures during the months of July and August, in addition to the lack of precipitation, were also a limiting factor for the growth and development of potato tubers. The number of trees per potato plant grown in the field in the two-year research and on three different land types was affected by the interaction of land type x year, while no influence of year was determined on plant mass, nor was the interaction of land type x year. The number of tubers and the average weight of the tubers were significantly influenced by the type of land and the year, while the influence of the interaction was highly significant. The yield of potatoes was significantly influenced by the type of land and the year, while the influence of the interaction was not significant. Potatoes grown in the Bijeljina locality (humofluvisol soil type) had the highest yields, and the lowest in Istočno Sarajevo (fluvisol soil type). Higher yields were achieved in 2018, compared to 2017, which was mostly influenced by the climatic and agroecological conditions at the time of the test. Favorable physical properties, primarily the mechanical composition, chemical and microbiological properties of the soil at the Bijeljina locality influenced the yield of potato tubers, so the highest yield was achieved at this locality.

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***Valentina KUNDIUS[1]***

# SOCIO-ECONOMIC EFFICIENCY OF THE APPLICATION OF NEW TECHNOLOGIES FOR THE PRODUCTION OF ORGANIC PRODUCTS

## SUMMARY

The article presents the results of scientific research on the effectiveness of new technologies for the production of organic food products, in particular biotechnologies, scientifically based crop rotation, the use of entomophages in biological plant protection, mechanical soil treatments in organic farming. The transition to organic agriculture is due to the negative consequences of global intensification for the environment, public health, and consumers of food products. The high nutritional value of organic agricultural products is shown. The efficiency of organic production is considered from the standpoint of a socio –ecological and economic approach. In the context of innovative development of organic agricultural and food production processes, points of economic growth are noted due to the introduction of biologization of agricultural production technologies, as a large-scale system program of the digital and technological generation, the development of which requires improvement of work with environmental resources, is a necessary condition for the development of organic production in agriculture. It is proved that the production of organic products in the regions improves the quality and standard of living of the population. The article substantiates the one-digit prospects for the production of organic agricultural products based on the use of agrobiological technologies, which combine the concept of AgroTech or high agro-industrial technologies and "Agriculture 4.0".

**Keywords**: organic products, agrotechnologies, biologization, efficiency of application

## INTRODUCTION

The concept of organic agriculture originated at the beginning of the twentieth century, when the need for food supply to the population in the countries of the world increased, which required solving the problem of soil depletion, insufficiency of crop varieties and the quality of agricultural and food

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products. At that time, the intensification of agriculture developed rapidly, which dramatically increased yields and made agriculture much more productive, intensive farming based on chemicalization, as a rule, yields higher yields (by about 5-34%) compared with organic farming (Seufert and Verena, 2012; Mazurova, 2022). However, the resulting negative consequences for the environment and public health stimulated the birth of the organic farming movement (Mazurova, 2022). The founder of organic agriculture abroad is Walter James, who in the 1940s first used this term in the book "Look at the Earth", and justified an ecological, natural approach to agriculture (Tarasov, 2007). Research in the field of farming without the use of chemicals was conducted by Rodale J., English botanist Albert Howard. In 1939, farmer Yves Belfort, influenced by the work of Albert Howard, sets up the world's first scientific experiment on agricultural land in Great Britain in order to compare organic and intensive agriculture (Lord Northbourne Walter James, 1939). Long before the global intensification, Russian scientists turned to organic agriculture. The founder of Russian agronomic science, A.T. Bolotov (1738-1833), formulated the basic principles of agroecology in 1771 in his work "On the division of fields" - farming in harmony with nature. His ideas and practical recommendations are actively used abroad in the production of organic products. Later, the issues of ecologization were developed in their writings by I.M. Komova (1750-1792), A.V. Sovetov (1826-1901), A.N. Engelhardt (1832-1893), P.A. Kostycheva (1845-1842), V.V. Dokuchaeva (1846-1903), I.A. Stebuta (1833-1923) and others (Tarasov, 2007).

Currently, the latest available data on organic agriculture worldwide shows that 2021 has been another good year for global organic agriculture (World Organic Market, 2021). According to the latest published FiBL study on organic agriculture worldwide, organic farmland and retail sales of organic products continued to grow and reached a new record high, as evidenced by data from (Willer *et al*., 2023). The area of organic farmland in the world has reached 72.3 million ha, increasing annually by an average of 10%. At the same time, the largest share of land for the production of organic products is in Oceania and Europe, the leader is Australia (35.69 million ha). The regions with the largest areas of organic agricultural land are Oceania (36.0 million ha – almost half of the world's organic agricultural land, i.e. 47 percent) and Europe (17.8 million ha, 23 percent) (data for the end of 2021). Globally, 1.6 percent of agricultural land is organic (Willer *et al*, 2023).The global organic food market is one of the fastest growing. Experts from various reputable organizations agree that it will continue to develop at a high pace. It is projected to increase sales of organic products to $ 212-230 billion by 2025 and replace 3-5% of the market for all agricultural products in the world. Already, almost 700 million people actively consume organic products. However, the concept itself is deeper than just safe nutrition (Willer *et al*, 2023).

The production of organic agricultural products, in the technological processes of which biomaterials, natural fauna, and agrotechnical techniques are used instead of chemical fertilizers, pesticides and fungicides (Federal Law, 2018), is being developed in the countries of the world as a segment and direction for improving modern agricultural production.

The negative consequences of global intensification over the decades have led to the need to change the theories of economic growth, when growth problems are considered inseparably from the concepts of "responsible consumption" in relation to nature and society. At the same time, qualitative indicators are more focused on the social component, assessment of health, active longevity, quality of life of the population, and food security. However, the decrease in crop yields with the abandonment of the use of mineral fertilizers, chemical plant protection products, and the underdevelopment of the organic market raise doubts among commodity producers and farmers about the expediency of switching to organic farming methods. At the same time, the problems of the development of organic agriculture are becoming urgent. Scientific research shows real opportunities to increase the efficiency of organic agriculture and its unambiguous prospects based on the use of biotechnologies, scientifically based crop rotations, agrotechnical techniques, organization of production and labor, cooperation and agro-industrial integration. In modern science and practice, agrobiotechnology is classified as a section of new technologies that combine the concept of AgroTech or high agro-industrial technologies and "Agriculture 4.0". In practice, it has been proven that agrobiotechnology increases the efficiency of agricultural production by 10 to 40 percent, reduces climatic stresses by 10 to 30 percent, increases soil fertility, and improves the quality characteristics of products (The Strategy, 2023).

Agriculture 4.0 represents a new evolution in the agricultural industry, combining advanced technolgies, innovative methods and sustainable resource management practices, including precision farming digitalization, automation and robotization of production processes, smart technologies in mechanization and electrification of production, artificial intelligence in agriculture, management of agricultural waste and climate change, sustainable rural development, the quality and safety of food products based on the production of organic products and the transition to a circular economy in the branches of the agro-industrial complex (Anishchenko and Shutkov, 2019; Panova, 2024). This concept provides not only an increase in production, an in-crease in resource productivity, but also environmental protection, which is of critical importance for ensuring food security in the face of a growing population and climate change, provides socio–ecological and economic benefits for the development of agriculture and rural areas.

In order to support the strategic development of organic agriculture, in order to meet the increasing demand for organic food products in the domestic and global markets, the Federal Law of the Russian Federation "On the Development of Agriculture" in Article 7 defines "The main directions of state support in the field of agricultural development", including "the development of organic agriculture and support for producers of organic products" (Federal Law, 2006).

The purpose of the scientific work, the results of which are presented in this article, is to generalize the theory and practice of replenishing soil fertility, the use of plant protection products and others, mechanisms for the development of modern organic agriculture based on the principles of responsible consumption. The scientific novelty of the work lies not only in substantiating the expediency, but also the effectiveness of the development of organic agriculture, the factors of its improvement from the standpoint of a socio–ecological and economic approach.

## MATERIAL AND METHODS

The research process uses a systematic approach, general scientific and empirical methods, including dialectical, review-theoretical, economic-statistical, computational-constructive, comparative and factor analysis, system-functional. In order to identify trends and problems in the development of organic agriculture, the positive experience of organic producers through review and theoretical methods, the materials of scientific research by Russian and foreign scientists on the forms and methods of organization and development of organic agricultural production in the Altai Territory were studied, tabular and graphical methods were used to visually present the results obtained, generalize and formulate conclusions about efficiency, methods of economic and statistical modeling and competitive analysis. With the help of an economic and statistical model, the forecast of grain yield and factors affecting it was carried out. The economic and statistical multifactorial model is represented by factors:

Y–grain yield, kg/ha;

$X_1$–applied to 1 hectare of mineral fertilizers, kg of active substance;

$X_2$–applied to 1 ha of organic fertilizers, tons.

It was assumed that the values of the resulting variable Y act as a function, the values of which are determined by the values of the explanatory variables acting as arguments to this function, so:

$Y = f(X1,\ldots,Xk)$

Among all possible functions $f(X1,...,Xk)$, a multiple linear regression model with a free term is selected (Shorokhova *et al.*, 2015).

The regression equation is obtained, which has the following form:

$Y = \beta_1 X_1 + \beta X_2 + \varepsilon$

Based on the obtained regression equation, it is possible to make a forecast of yield per hectare of acreage, while there was no close relationship between the yield of grain crops and the amount of mineral and organic fertilizers applied to cereals in the Altai Territory, therefore, this factor may be a prerequisite for the development of agriculture focused on the production of organic products.

## RESULTS AND DISCUSSION

It is based on the principles of the "green economy", which implies a responsible human attitude to the natural resources of the planet and a compromise between increasing production and preserving their potential. Organic products are food products grown or manufactured without the use of synthetic pesticides, synthetic fertilizers, biological and chemical (again synthetic) additives (antibiotics, growth hormones and others), as well as genetically modified organisms of plants or animals. These requirements are valid for both agricultural products and food industry products. Studies have shown that there are no big differences in the content of minerals and vitamins in conventional and organic products. “Organic” meat contains more polyunsaturated fatty acids and omega-3 compared to regular meat, although these comparisons are not entirely correct due to the heterogeneity of the samples studied.

The nutritional composition of milk and dairy products may vary depending on the components of the feed, the time of year, etc. However, milk produced both “organically” and traditionally raised cows has the same amount of protein, vitamins, lipids and trace elements. A study conducted in England demonstrated that “organic” milk has significantly higher levels of polyunsaturated fatty acids (including conjugated linoleic acid and alpha-linolenic acid), alpha-tocopherol and iron than conventional, but lower levels of iodine and selenium (“Organic” products. Nutritional value, 2023). The contribution of organic agriculture to comprehensive sustainable development strategies such as the Sustainable Development Goals and the European Un-ion's Farm-to-Fork Strategy. In Russia, a modern approach to the production of organic products is being formed in accordance with the application of Federal Law No. 280-FZ from January 1, 2020, as well as a strategy for the development of organic production until 2030. In the coming years, the Russian organic market will continue to grow at an average rate of 10-12% per year, and its volume by the end of 2022 may amount to more than 14 billion rubles (Organic market, 2023).

In the Altai Territory, as our research has shown, there is sufficient potential for the production of organic products, but only 7 producers of agricultural and food products have passed certification procedures to con-firm the status of "Organic". The most significant of them is Stepnoy LLC of Biysk district, which has been operating using organic technologies since 2008 without applying fertilizers, without using chemical plant protection products against weeds, pests and diseases. The farm specializes in the cultivation of cereals and legumes (buckwheat, rye, oats, spring and winter wheat, peas, rapeseed). The cultivated area is more than 6,000 hectares. Half of the fields are occupied by buckwheat, the rest by oats, peas and wheat. The agricultural enterprise works in cooperation with the certified processor of organic products LLC Kurai Agro Plus, LLC Predgorye, which sells organic products, a specialized organic food store has been opened in Biysk and others. As follows from the data in Table 1, the yield of cereals and buckwheat is not much lower in the farm compared to the average in the Biysk region, oats are 1-2 hundredweight higher per 1 hectare.

A comparison of the costs of grain and leguminous production using organic technologies in Stepnoy LLC and industrial methods in Prigorodnoye Educational and Experimental Farm JSC of the Altai Territory shows that with significant costs for the application of mineral fertilizers and the use of chemical plant protection products, the yield of cereals and leguminous crops in Prigorodnoye Educational and Experimental Farm JSC" 1.4 – 1.5 times higher, the cost of a unit of production is 1.2 -1.05 times lower. In order to assess the economic efficiency of intensive and organic crop cultivation technologies, economists have calculated technological maps (Vinnichek and Zaruk, 2022).

Table 1. Yield of main crops, kg/ha in LLC "Stepnoy" (household) Biysk district in comparison with the average indicators for the Biysk district (district) of the Altai Territory

| | 2017 | 2018 | 2019 | 2020 | | 2021 | | 2022 | 2023 |
|---|---|---|---|---|---|---|---|---|---|
| The indicators | "Stepnoy" | "Stepnoy" | "Stepnoy" | "Stepnoy" | Biysk district | "Stepnoy | Biysk district | "Stepnoy" | "Stepnoy" |
| Winter wheat | 10,3 | 27,1 | 32,2 | 19,3 | 23,1 | 26,5 | 26,9 | 22,2 | 14,8 |
| Spring wheat | 24,0 | - | 14,5 | 13,7 | 20,9 | 21,0 | 25,3 | 28,3 | 25,9 |
| Barley | 27,5 | 21,0 | 20,9 | 18,4 | 19,8 | 13,0 | 26,6 | 15,0 | 9,1 |
| Oats | 27,1 | 31,1 | 30,7 | 17,5 | 15,0 | 21,0 | 18,8 | 20,9 | 26,1 |
| Buckwheat | 10,2 | 10,0 | 7,9 | 10,1 | 11,6 | 8,3 | 10,2 | 9,5 | 13,4 |

The technology assessment was carried out based on the condition that agricultural crops are placed on soils of the first group (PH - 5.6 and above, $P_2O_5$ - 100 and above, $K_2O$ - 80 and above) for a more objective comparison of the economic results of each of the three technologies (Table 2).

Table 2. Efficiency of various technologies of spring grain crops cultivation

| Indicators | Spring cereals with sowing of perennial grasses | | |
|---|---|---|---|
| | Intensive technology | Organic technology (Model - 2) | Organic technology (Model - 1) |
| Yield, centner /ha | | | |
| basic production | 28 | 25,2 | 22,4 |
| by-products (related) | 22,4 | 20,16 | 17,92 |
| The total cost of production, thousand rubles/ha. | 19,22 | 19,89 | 21,53 |
| Expences per 1 ha, thousand rubles, cost price 1 centner, rubles | 12,69 | 10,03 | 9,37 |
| basic production | 407,99 | 358,10 | 376,34 |
| by-products (related) | 56,67 | 49,74 | 52,27 |
| Conditional net income, thousand rubles/ha. | 6,53 | 9,86 | 12,16 |
| Profitability, % | 51,41 | 98,38 | 129,81 |

Thus, the economic benefits of organic agriculture have also been proven. Modern scientific research by agricultural scientists is aimed at developing agrotechnologies that make it possible to replenish soil fertility, protect plants from pests, and animals from diseases. In a discussion organized by the Union of Organic Farming, scientists and practitioners noted that Russia needs to stop and not go into a dead end of chemicalization. Russian science is ready to replace 40-50% of imported agro-pesticides with environmentally friendly biological products (National Organic Union, 2023). The economic agro-industrial policy is aimed at ensuring stable growth in the production of agricultural ecological products, protection and technological use of land, and increasing the effectiveness of the introduction of biologization.

Our research on the effectiveness of the use of expensive mineral fertilizers in agricultural organizations of the Altai Territory showed a low dependence of increasing the yield of grain and leguminous crops on the amount of fertilizers applied based on the construction of a multifactorial economic and statistical model reflecting the dependence of grain yields on the level of application of mineral and organic fertilizers and solving an economic and mathematical problem. The economic and statistical multifactorial model is represented by factors:

$y$ – grain yield, kg/ha;

$x_1$ – applied to 1 hectare of mineral fertilizers, kg of active substance;

$x_2$ – applied to 1 ha of organic fertilizers, t

Data analysis in the period 1966-2021 (Table.3) showed that there is a weak direct relationship be-tween grain yield and the volume of application of mineral fertilizers per 1 hectare in the Altai Territory, the correlation coefficient r=0.29.

There is a weak direct relationship between grain yield and the application of organic fertilizers per 1 ha of crops, since the correlation coefficient r=0.23, with an increase in the dose of organic fertilizers per 1 ha of sowing, the yield of grain crops will increase.

The regression equation has the following form:

$$y= 12{,}199 + 0{,}1738x_1 – 3{,}321x_2$$

Based on the obtained regression equation, a yield forecast can be made, which will amount to 1.52 tons/hectare. The actual yield of grain crops in the Altai Territory was, respectively, 1.69 and 1.38 tons /hectare in 2022 and 2023 (Altai Territory, 2024). In general, as a result of the study, there was no close relationship between the yield of grain crops and the amount of mineral and organic fertilizers applied in agriculture in the Altai Territory, therefore, this factor may be a prerequisite for the development of agriculture focused on the production of organic products and the abandonment of the use of mineral fertilizers in agriculture. Due to their low efficiency in the conditions of agricultural production in the Altai Territory.

In general, it should be noted that there is no close relationship between the yield of grain crops and the amount of mineral and organic fertilizers applied in agriculture in the Altai Territory, therefore, this factor may be a prerequisite for the development of agriculture focused on the production of organic products and the abandonment of the use of mineral fertilizers due to their low efficiency in agricultural production Altai Territory. To create an effective system for using the region's resources until 2035, the Altai Territory adopted the law "On Approval of the strategy for socio-economic development of the Altai Territory", which creates conditions for the development of measures to support the production of eco-products.

The Altai Territory has absolute competitive advantages for the development of this area, including land, human resources and technological resources. Technologies for the production of organic crop production using biological products have been developed.

Table 3. Yield and availability of crops of grain crops with organic and mineral fertilizers in the Altai Terri-tory for the period 1966-2021.

| Years | Per 1 ha of crops | | Yield, kg/ha |
|---|---|---|---|
| | mineral kg D.V. | Organic, t | |
| 1966-1970 | 5,7 | 0,4 | 10,5 |
| 1971-1975 | 10,0 | 0,8 | 13,3 |
| 1976-1980 | 18,2 | 1,2 | 11,4 |
| 1981-1985 | 27,7 | 1,6 | 11,0 |
| 1986-1990 | 38,0 | 1,7 | 13,4 |
| 1991-1995 | 11,2 | 0,8 | 10,2 |
| 1996-2000 | 2,1 | 0,2 | 9,1 |
| 2001-2005 | 1,6 | 0,2 | 11,8 |
| 2006-2010 | 2,9 | 0,2 | 12,6 |
| 2015 | 6,8 | 0,3 | 14,3 |
| 2018 | 11,9 | 0,3 | 15,6 |
| 2019 | 15,3 | 0,2 | 14,6 |
| 2020 | 23,4 | 0,2 | 12,6 |
| 2021 | 23,6 | 0,2 | 17,3 |

The branch of the Federal State Budgetary Institution "Rosselkhoznadzor for the Altai Territory and the Altai Republic" is increasing the production of biological products such as "Humate +7B", "Pseudobacterin -2, Zh", Rizoplan, Rizotorphin (Fig. 1) due to the increase in their demand. In 2020 - 2021, the branch expanded its range with new drugs, such as Pseudobacterin-2, Zh, Azolene.The profitability of the branch in 2022 amounted to 21%. It is proved that the use of biological products in agriculture contributes to an increase in productivity and product quality against the background of cost reduction and increased profitability of production. The heads of organizations producing organic products point to a number of constraining factors, the main one is the high cost of certification of farm lands and products. Therefore, the process of entering organic agriculture is lengthy and requires government support and preferences (Official website of the Altai Territory, 2024).

In order to make better and more effective use of internal capabilities in solving the problems of biologization of production, we have proposed the activation of marketing activities in this direction, the expansion of the network of consulting points to provide advice to rural enterprises, entrepreneurs, and individuals.In the activity of the Rosselkhoznadzor, it is important to take into account the uniqueness of the conditions of each region and understand that there are no universal solutions in the biologization of organic production.

Therefore, the popularization of biological methods of plant protection occupies an important place in the activities of the Federal State Budgetary Institution "Rosselkhoznadzor" (Bulletin of the Rosselkhoznadzor, 2022), which is especially important in conditions of high demand for organic products.

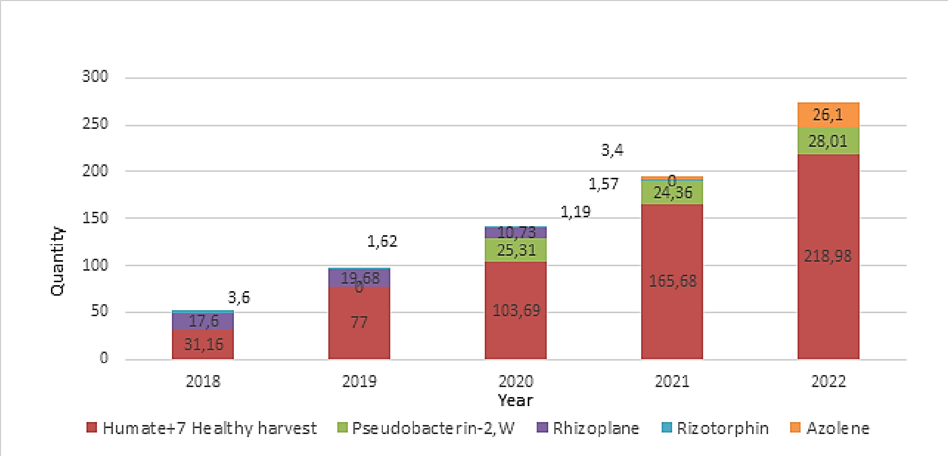


Figure 1. Dynamics of the production of biological products of the branch of the Federal State Budg-etary Institution "Rossel-Khozcenter" in the Altai Territory and the Altai Republic 2018-2022.

According to various authors, application of natural compounds, such as plant extracts and oils, use of antagonistic microorganisms can be used in crop protection practices, and facilitate high levels of disease control and production yield (Golijan-Pantović *et al.* 2022). Joint research and development of organic farming technologies have shown that as a result of the introduction of rhizobacterial fertilizers developed by scientists in Mongolia, the harvest of spring wheat increased by 11.3-46.9%, seed potatoes - by 20-65%, various vegetables such as cabbage, carrots, cucumbers and turnips - by 22.2- 83.6%. The use of Azophos biofertilizer in Seleng Aimag allows to increase the yield of all types of agricultural crops by 1.5-2.0 times. The use of microorganisms and biostimulants can increase crop yields by 20-30%, and also compensates for about 30% of nutrient losses from the soil. 1 liter of the new biological product Mongol EM replaces 20-40 kg of mineral fertilizers. A new type of biopreparation Mongol EM turned out to be safe for food, harmless to human health, it can be used to clean polluted lands and waters, production and introduction of biopreparations for plant protection (Kundius *et al.*, 2020).

According to scientists, agriculture is approaching the threshold moment when a new wave of technologies will provoke a revolution comparable to the mechanization of industry in the 20th century, which will inevitably lead to an increase in labor productivity (Anishchenko and Shutkov, 2019; Panova, 2024). Scientific developments of Altai scientists (FGBNU FANTSA) offer new, locally adapted, high-yielding (6.0-9.0 tons/hectare), diseaseresistant varieties of cereals, fruit and berry crops, and antiparasitic drugs for animals, applicable in organic agriculture. ASAU conducts scientific research on replenishing and increasing soil fertility using siderates, legumes and other useful crops in crop rotations, mechanical treatments, the use of entomophages for plant protection, effective feed additives, medicinal preparations for organic livestock and others. Research has shown that for small businesses, the production of organic products can be a window of opportunity that allows them not just to survive, but to fully compete due to high product quality (Kundius, 2020; Kundius and Bayarsukh, 2023; Kundius *et al.,* 2024).

In the context of the transition to the next evolutionary stage of technical and technological development – "Agriculture 4.0", new precision farming technologies are being developed and applied in the agriculture of the region. Precision agriculture works by using data from various sensor technologies to improve the efficiency of all types of work. Intelligent sensors of agricultural crops analyze parameters such as humidity, soil electrical conductivity, humus height, and the content of organic substances in the soil. Unmanned aerial vehicles in agriculture equipped with appropriate sensors (video cameras, infrared, multispectral, hyper-spectral cameras and others) allow for the assessment of the condition of crops; identify pests; to determine the lack of moisture; to analyze the condition of the soil; to map; to treat crops with appropriate biological products; to check irrigation systems and much more. Artificial intelligence is being used in organic agriculture, which can process and analyze images from drones or stationary cameras and, on this basis, detect plant diseases, the presence of pests or nutrient deficiencies in the early stages; It can also analyze data on soil moisture, weather conditions and plant needs and optimize the irrigation regime, thereby reducing re-source consumption, increasing the efficiency of organic technologies.

## CONCLUSIONS

Thus, taking into account the social, environmental and economic effects, organic agriculture is undoubtedly more efficient. Efficiency is achieved and increased through the use of agrobiotechnologies, techniques for improving agricultural landscapes, and biologization of the technological cycle, which scientists from many countries and regions of Russia are working on creating and improving (Russian Agricultural Biotechnologies, 2024). In the context of international sanctions, the tasks of reducing the level of import dependence and, accordingly, regional problems of organic production in agriculture remain relevant in Russia. At the same time, there is a need to develop regional development strategies that will contribute to increasing the efficiency of the use of biologization of production and economic transformations in the agricultural sector of the region, including through the effective use of organic production technologies.Research has shown that for small businesses, the production of organic products can be a window of opportunity, allowing them not just to survive, but to fully compete due to the high quality of products. State support is important for organic producers, especially at the stage of formation. The adoption of appropriate legislative and regulatory acts will allow the Russian market of organic products to successfully grow and develop.

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***Gordan S. KARAMAN*** [1]

# THE SUBTERRANEAN FAMILY NIPHARGIDAE (CRUSTACEA) IN BULGARIA (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 339)

## SUMMARY

The family Niphargidae (Crustacea Amphipoda Senticaudata) in Bulgaria is studied, synonymy, new and known localities of each species are presented. Holotype and paratype of *Niphargus vlkanovi* S. Karaman & G. Karaman, 1959 (cave near Zhivata voda) is partially redescribed and figured based on existing slides. The new subspecies *N. vlkanovi burgasi,* ssp. nov. from several localities from Burgas region is described and figured, and its relation to the ssp. *vlkanovi* is discussed. Some new data of *N. bureschi* Fage, 1926, *N. toplicensis* and *N. georgievi* S. Karaman & G. Karaman, 1959 are presented and locus typicus of *N. georgievi* is established. Key to the *Niphargus* species of Bulgaria (18) is composed.

**Keywords**: taxonomy, subterranean Crustacea, Amphipoda, *Niphargus*, *burgasi*, key, Bulgaria.

## INTRODUCTION

The fauna of the subterranean family Niphargidae (Crustacea: Amphipoda) is only partially investigated in Bulgaria by various authors. First mentioned species from Bulgaria cited Schäferna (1922) sub name *Niphargus tatrensis* from Vitosha Mt. 800 m asl. (probably *N. vlkanovi*).

French scientist L. Fage mentioned (1926) *N. puteanus* (Koch) from Progled Cave, Ćepelare district, Rhodopes Mts., 1000 m asl., S. Bulgaria. This locality was mentioned later by Andreev (1972) and Beron et al. (2011) as Sbirkovata peshtera Cave (Sm 4) (= *N. cepelarensis* S. Karaman & G. Karaman, 1959).

Fage described (1926) also first new species from Bulgaria, *Niphargus bureschi,* n. sp. from Dupka Cave near Zakatnik (=Lakatnik), Golemata peštera Cave near village Micre and Divitaška peštera Cave, in Lowetch region. Later Stanko Karaman & Gordan Karaman described (1959) some new species

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(*pecarensis, georgievi, cepelarensis, vlkanovi*) from various localities of Bulgaria, mentioning several new localities of *N. bureschi*.

Andreev (1966) described *N. toplicensis* n. sp. and (1972) cited some new localities of *N. bureschi*. G. Karaman (1973) mentioned *N. valachicus* from Devnja. Andreev (2001) described new species *N. bulgaricus,* and Kenderov & Andreev (2015) described new species *N. cvetkovi*. The species *Niphargopsis trispinosus* Dancau & Capuse, 1959 [loc. typ.: Mehadia, Romania] was cited by Andreev (2001) from Bulgaria. G. Karaman (2022) cited *Niphargus decui* G. Karaman & Sarbu, 1995 from Shabla, .and later (2024b) cited *Niphargus kragujevensis remus* G. Kar. 1992 and *N. kragujevensis femineus,* ssp. nov. from Bulgaria. In the present work is described a new subspecies, *N. vlkanovi burgasi,* ssp. nov. from Burgas region.

The further investigations probably will elevate the number of known subterranean taxa of family Niphargidae in Bulgaria.

## MATERIAL AND METHODS

The collected material was preserved in the 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All appendages were temporarily submersed in the mixture of glycerin and water for study and drawing. After study, all appendages are transferred to Liquid of Faure on permanent slides.

All illustrations were inked manually. Some morphological terminology and setal formulae of mandibular palpus and gnathopods 1-2 -propodus follows Karaman`s terminology (Karaman, G. 1969; 2012). All studies in this work are based on the classic morphological, ecological and zoogeographical studies. Terms “setae” and “spines” are used based on its shape, not origin.

## TAXONOMICAL PART
## Order AMPHIPODA
## Suborder SENTICAUDATA Lowry & Myers, 2013
## Family NIPHARGIDAE

### *NIPHARGUS BULGARICUS* Andreev, 2001

*Niphargus bulgaricus* Andreev, 2001: 79, figs. 1-3; Vidinova et al., 2016: 153.

**LOCUS TYPICUS:** Lake Bolata, N. of cap Kaliakra, Varna reg.), Bulgaria.

**LOCALITIES CITED**: BULGARIA

Andreev, 2001: Lake Bolata, cap Kaliakra, village Kavarna, reg. Varna; Fontain near river

Ropotamo v. Primorsko, reg. Burgas (NE Bulgaria).

Vidinova et al., 2016: Shabla Lake [43$^{0}$34`32.2”N, 28$^{0}$34`01.8”E].

**DISTRIBUTION:** Bulgaria, endemic.

## *NIPHARGUS BURESCHI* Fage, 1926
Figs. 10 E-H

*Niphargus plateaui bureschi* Fage, 1926: 24, figs. 1-18; Schellenberg, 1934: 209;

*Niphargus bureschi* Schellenberg, 1935: 208 (key); S. Karaman & G. Karaman, 1959: 158; Barnard, J.L & Barnard, C.M., 1983: 690; Andreev,1972: 65; G. Karaman & Ruffo, 1986: 523; Pandourski, 1993: 165; Beron, 1994: 48; Beron et al., 2011: 591.

LO**CUS TYPICUS**: This species was described by Fage from several localities, among them Temnata Dupka Cave near Lakatnik, Bulgaria. As locus typicus and holotype were never selected, we selected now the locus typicus, Temnata Dupka Cave near Lakatnik, Bulgaria. In the case that Fage`s material of *N. bureschi* is not preserved, we proposed lectotype (male 19 mm) (No.S-1099A) and paralectotype (female 15 mm) (No. S-1099B). They are temporarily deposited in Karaman`s Collection in Podgorica, Montenegro.

**MATERIAL EXAMINED**: BULGARIA:

IP-21= Dineva peštera Cave near village Gingji, Godečko, Stara planina Mts., 24.3.1995, 2 exp. (leg.?);

IP-23=“Dinevata Cave”, village Ginzi, dept. of Sofia, Stara planina Occidentale Mt.,1000 m asl., flaques, 28.11.1995, 1 exp. (leg. ?);

X-632- Dineva peštera Cave near village Gingji, Godečko, 12.7.1940, 2 exp. (leg.?);

X-633= Golema peŝtera near village Micre, Lowetch, 4.9.1924, 1 exp. (leg. Deltchev);

X-634= Temnata Dupka Cave near Lakatnik, 16.2.1958, 1 exp. (leg. V. Georgiev);

S-1099= Temnata Dupka Cave near station Lakatnik, 16.11.1932, 3 exp. (leg. Ivanov);

S-6789= Western Rhodopes Mts., Dobrostan Mt., Martsin gamtsa, cave Ivanova Voda, alt. 1320 m asl., ungerground lake, 8 exp. (leg. B. Petrov & N. Simon);

BU-4= Village Bohot, district Pleven, gouffre Kirov Vartop, 6.4.1985, 3 exp. (leg. P. Beron);

BU-9= Cave Leleška Dupka, village Iglika, district Jowbol, 1 exp. (leg. Beron & Beškov).

**LOCALITIES CITED:** BULGARIA:

Fage, 1926: Dupka Cave near Zakatnik (=Lakatnik), Kodja Balkan; Golemata peštera Cave near village Micre (Lovetch reg.); Divitaška peštera Cave, Lovetch reg.

S. Karaman & G. Karaman, 1959: Dineva peštera Cave near village Gingji Godečko; Temna dupka Cave, Lakatnik, 420 m asl.; Ptičata dupka Cave on

Stara planina Mts. near Trojan Bulgarie; Cave Lepenica, Čeninsko (Pazardzhik reg.); Zadanka Cave near Karlukovo; Golemata peštera Cave near village Micre; Divitaška peštera Cave near town Lovetch.

Andreev, 1972: Cave Goliami petch near village Varbovo, mixed with *N. pecarensis*; Dineva peŝtera Cave (Stara planina Mts.). Cave "Lepenitza"(Rhodopes Mts.).

Beron, 1994: Elata (Sf. 48); Pandourski (1992a: 404), Levica spring (Mt 2); Vreloto (Mt 3); Propast (Vd 7); Vodni petch (Vd 15); Raenkov kladenec (Vd 26); Jame 1 (Vd 27); Zmiiskata propast (Vd 44); Falkovskata peštera Cave (Vd 46); Jamata (Sf 15); Vodnata peštera Cave (Sf 52); (Pandourski, 1993: 165);

Beron, Petrov & Stoev, 2011: Lepenica Cave near Velingrad (Pazardzhik region).

**DISTRIBUTION**: Bulgaria, endemic.

**REMARKS.** Fage described and figured well this species. Variability of some morphological characters has discussed by S. Karaman & G. Karaman (1959) as well as by Andreev (1972). We mentioned some remarks only.

This species was characterized by specific shape of telson remarkably tapering distally, but in some localities this shape is rather modified, lobes are more obtuse and with rather longer distal and marginal spines. The number of telson spines is variable (3-5), along inner (mesial) margin of lobes appear 0-3 short spines or spine-like setae, along outer margin 1-3 spines. Telson is usually always without facial spines, but sometimes one short facial spine can occur on one or both lobes (Fig. 10G, H).

Epimeral plates are always slightly pointed or sharply angular, with various numbers of ventral spines.

Urosomal segments 1-2 with rather variable dorsal spinulation, urosomal segment 1 usually with 1 spine and 1 seta, on each dorsolateral side; urosomal segment 2 with 1-3 spines and 0-1 seta on each dorsolateral side.

Maxilla 1 inner plate with 4-6 distal setae, palpus not reaching distal tip of outer plate-spines (Fig. 10E). Maxilliped inner plate not exceeding outer tip of first palpus article, with up to 6 distal spines (Fig. 10F), palpus article 4 with 2 mesial setae near basis of the nail.

Gnathopod 1 in males remarkably smaller than gnathopod 2, both gnathopods with article 3 bearing one distoposterior bunch of setae on article 3. Propodus of gnathopods 1-2 trapezoid. Palm of gnathopod 1 inclined up to half of propodus-length, palm of gnathopod 2 inclined over half of propodus-length; the shape of propodits can be rather variable according the age and sex. Palm is defined on outer face by corner S-spine accompanied laterally by 2-3 L-spines and 4-5 corner facial M-setae, on inner face by one subcorner R-spine, dactylus with several bunches of 1-3 setae along outer margin.

Dactylus of pereopods 3-7 more or less strong, with strong or weak spine at inner margin. Article 2 of pereopods 5-7 longer than broad, without ventroposterior lobe.

Pleopods with 2 retinacula, peduncle of pleopods scarcely setose, usually with only 0-3 setae each.

Uropod 1 peduncle with dorsoexternal row of spines and dorsointernal row of setae; rami in males and females of nearly equal length.

Uropod 3 elongated in males, with outer ramus composed of 2 nearly equal articles in adult specimens; in females outer ramus of uropod 3 with second article much shorter than first one.

Large distribution area of this species in Bulgaria and variation of many morphological characters suggest necessity for the further studies of variability of its morphological characters.

### *NIPHARGUS CEPELARENSIS* S. Karaman & G. Karaman, 1959

*Niphargus cepelarensis* S. Karaman & G. Karaman, 1959, 153, figs. 17-23; Andreev, 1972: 64; Barnard, J.L. & Barnard, C.M., 1983: 690; G. Karaman & Ruffo, 1986: 524; Beron, 1994: 13, 486; Beron, Petrov & Stoev, 2011: 591.

*?Niphargus puteanus* Fage, 1926: 6.

**LOCUS TYPICUS**: Peštera Cave near village Progled- Čepelare, Rhodopes Mts., 1000 m asl., S. Bulgaria. Andreev (1972) and Beron et al. (2011) cited this locality as "Sbirkovata peštera Cave near Progled Village (Smolyan reg.), 1430 m. asl. In the same cave Beron et al. (2011) mentioned also *N. puteanus*.

**MATERIAL EXAMINED**:

Holotype Slide II/9

**LOCALITIES CITED: BULGARIA**:

Fage, 1926: Progled Cave, Ćepelare district, Rhodopes Mts., 1000 m asl., S. Bulgaria (sub name *N. puteanus* (Koch.).

S. Karaman & G. Karaman, 1959: loc. typ.

Andreev, 1972: locus typicus.

Beron, 1994: Bulgaria: (no data).

Beron, Petrov & Stoev, P., 2011: Sbirkovata peštera Cave near Progled Village (Smolyan reg.), 1430 m. asl., Temp. $6^0$C. [In the same cave he mentioned also *N. puteanus*].

**DISTRIBUTION**: Bulgaria, endemic.

**REMARKS**. This species was described based on females only. Andreev (1972) collected the males also. He mentioned inner plate of "maxillullae" 1 of males is with 2 spines; nail of pereopod 7 dactylus is longer than half of dactylus; uropod 3 in male with articles of outer ramus of equally length, in females distal articles ¼ shorter. He mentioned that established differences don't change the taxonomical position of this species. Unfortunately he omitted to describe uropod 1 in male.

**DISTRIBUTION:** Bulgaria, endemic.

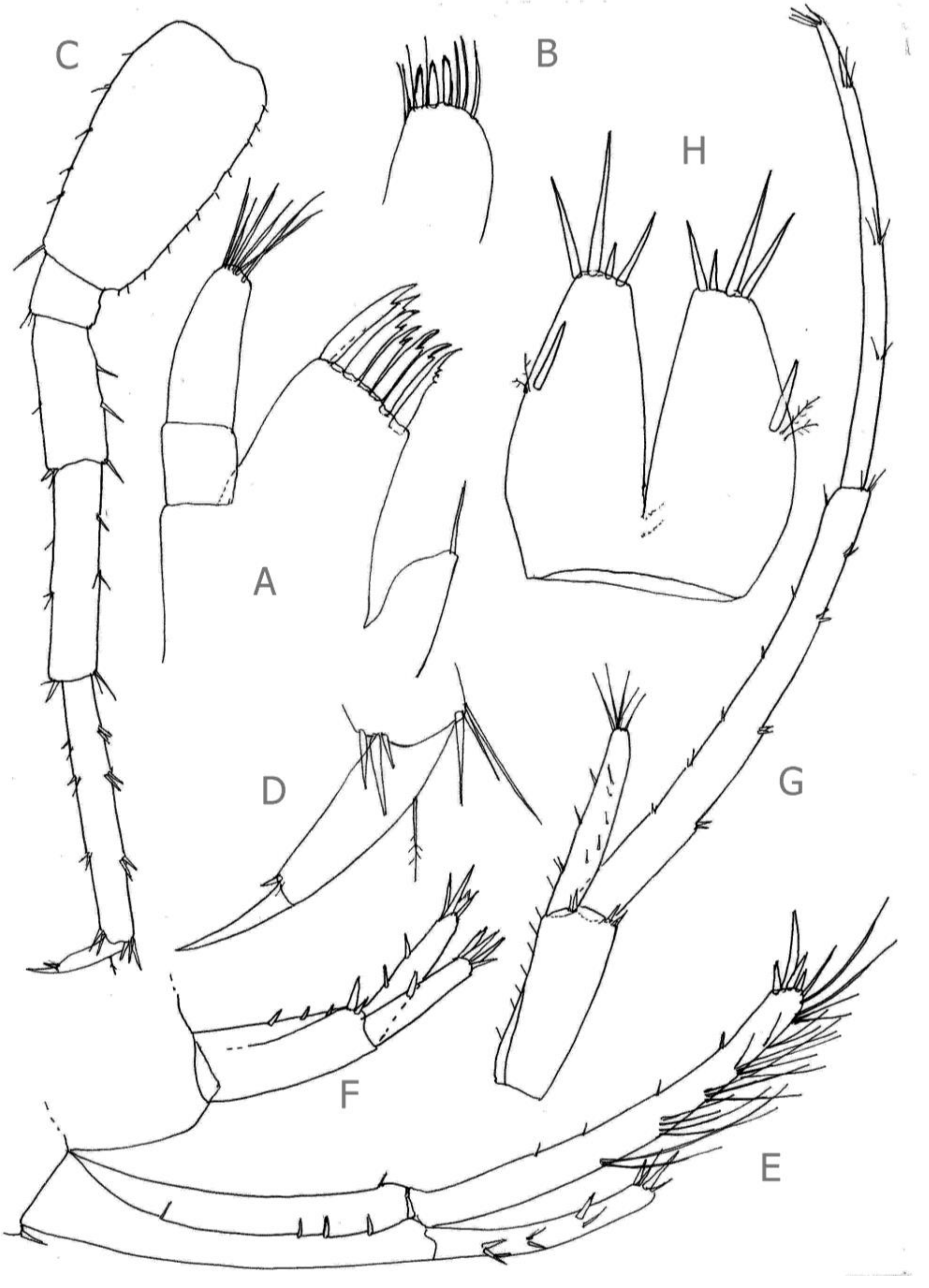


Fig. 1. *Niphargus vlkanovi vlkanovi* S. Karaman & G. Karaman 1959, cave near Zhivata Voda, holotype slide, male 12.0 mm,: A= maxilla 1; B= maxilliped, inner plate; C= pereopod 7; D= dactylus of pereopod 7; E= uropod 1; F= uropod 2; G= uropod 3; H= telson.

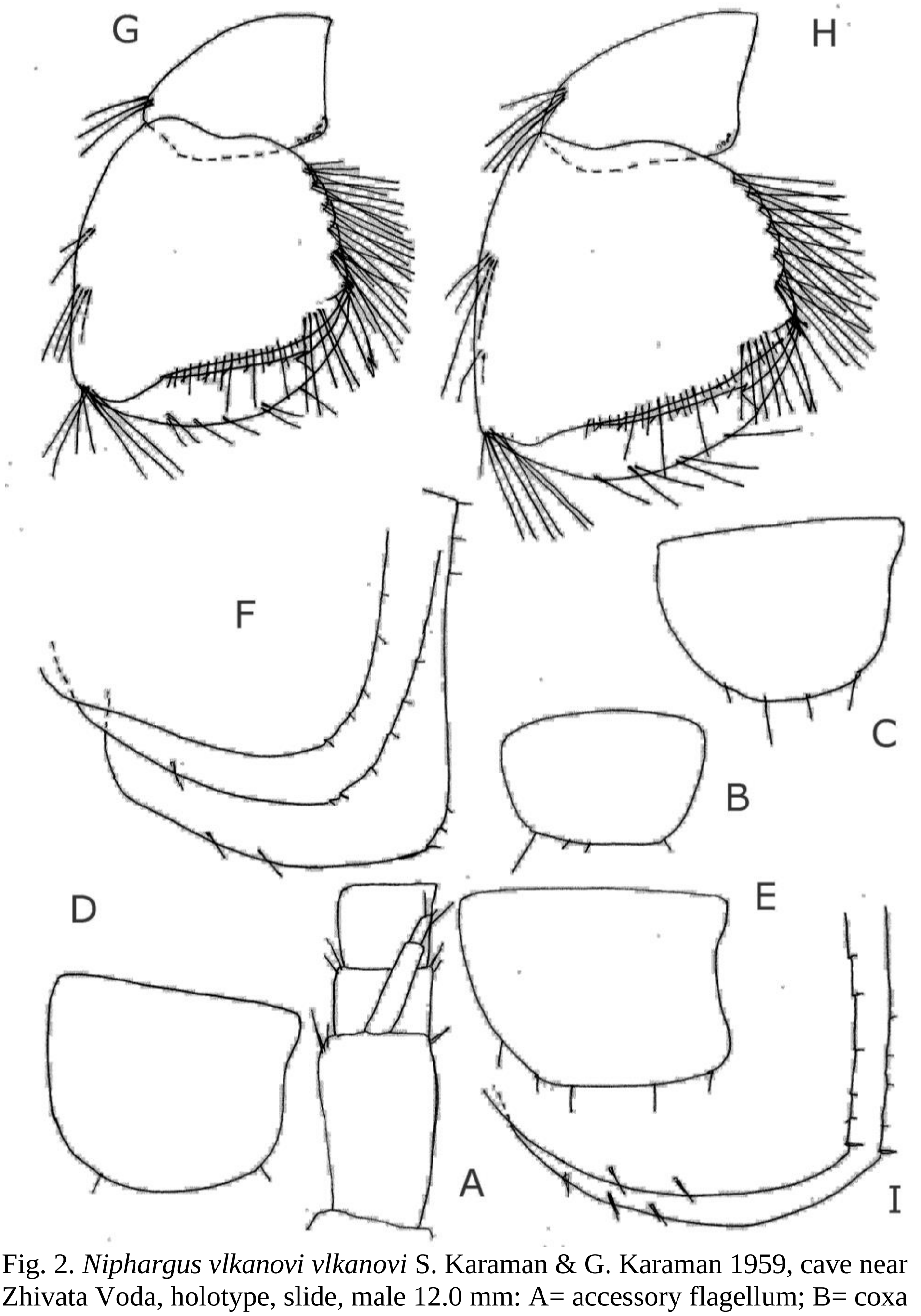


Fig. 2. *Niphargus vlkanovi vlkanovi* S. Karaman & G. Karaman 1959, cave near Zhivata Voda, holotype, slide, male 12.0 mm: A= accessory flagellum; B= coxa 1; C= coxa 2; D= coxa 3; E= coxa 4; F= epimeral plates 1-3; G= gnathopod 1; H= gnathopod 2.

**Female paratype 7.0 mm**: I= epimeral plates 1-3.

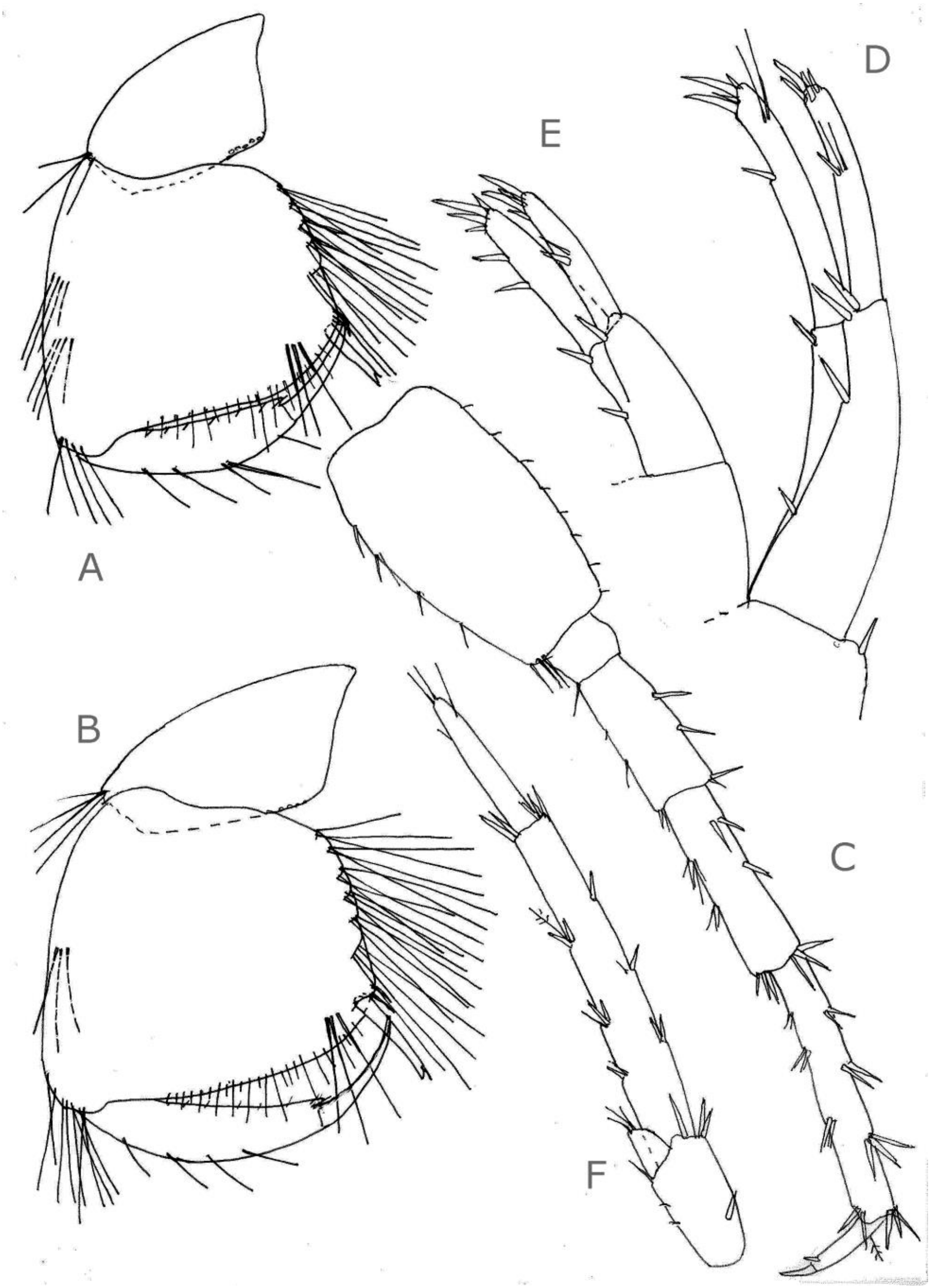


Fig. 3. *Niphargus vlkanovi vlkanovi* S. Karaman & G. Karaman 1959, cave near Zhivata Voda, paratype, slide, female 7.0 mm: A= gnathopod 1 propodus; B= gnathopod 2 propodus; C= pereopod 7; D.= uropod 1; E= uropod 2; F= uropod 3.

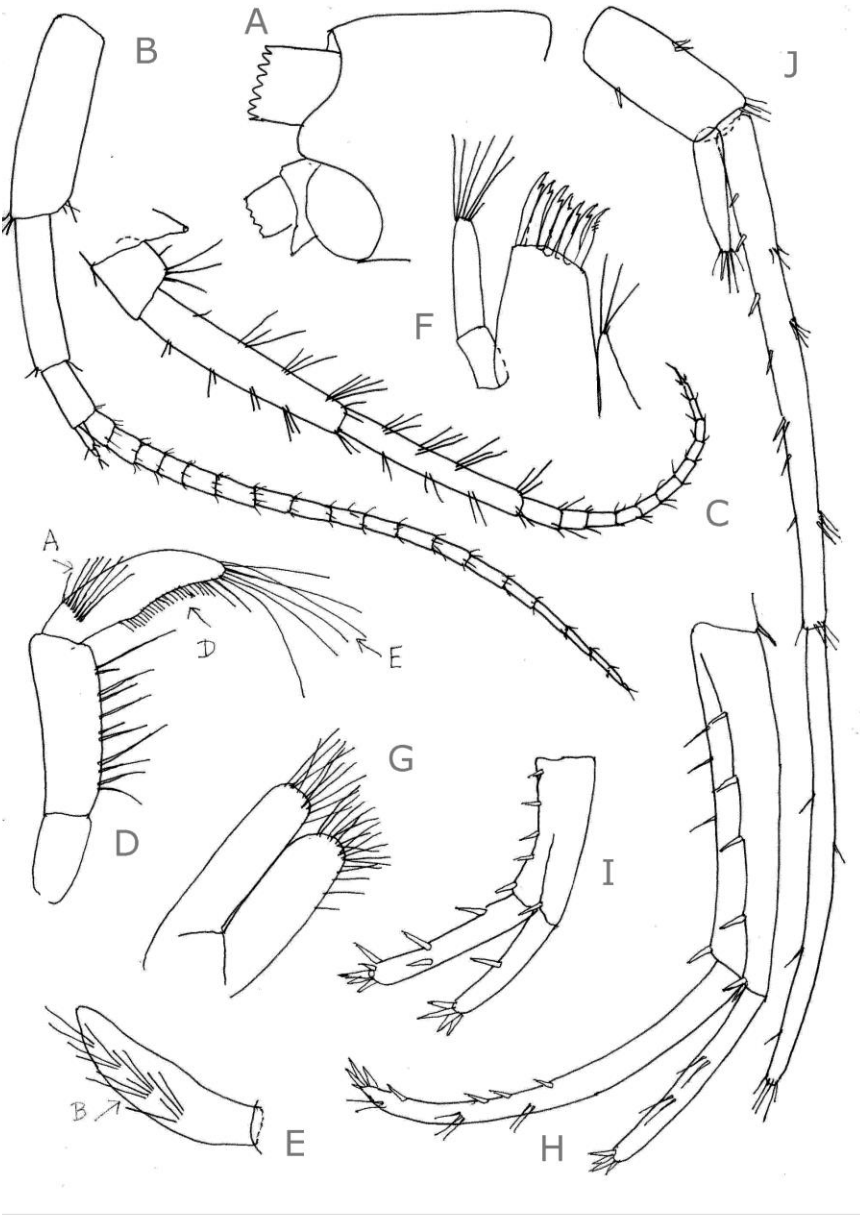


Fig. 4. *Niphargus vlkanovi burgasi*, ssp. nov., Verovnik, Burgas, male 15.5 mm (holotype): A= head; B= antenna 1; C= antenna 2; D= mandibular palpus, outer face (A= facial A-setae; D= lateral marginal D-setae; E= distal E-setae); E= last mandibular palpus article, inner face (B= facial B-setae); F= maxilla 1; G= maxilla 2; H= uropod 1; I= uropod 2; J= uropod 3.

### *NIPHARGUS CVETKOVI* Kenderov & Andreev, 2015

*Niphargus cvetkovi* Kenderov & Andreev, 2015: 179, figs. 1-4; G. Karaman, 2024b: 15.

**LOCUS TYPICUS**: Water source "Cheshma Gorgoritsa" near the village Novi Han, E. of Sofia, Bulgaria.

**LOCALITIES CITED**: Known from locus typicus only.

**DISTRIBUTION**: Bulgaria, endemic.

**REMARKS:** Kenderov & Andreev (2015) well described this species. The pilosity of peduncles in pleopods 1-3 is not mentioned, and the figured uropod 1 in male (Fig. 4b) seems to be with rather inflated rami, although authors don`t mentioned it in its description of this species.

### *NIPHARGUS DECUI* G. Karaman & Sarbu, 1995

*Niphargus decui* G. Karaman & Sarbu, 1995: 77, figs. 1-5; Karaman, G., 2022: 107, figs. 1-6;

? *Niphargus* sp. Andreev, 2001: 85.

**LOCUS TYPICUS**: Vama Veche village, 10 km south of Mangalia, well, Romania.

**MATERIAL EXAMINED**:

BULGARIA:

BU-11= Shabla, Tolbuhin region [nearly 65 km NE of Varna], sondage, 3.11.1978, 10 exp. (leg. L. Cvetkov).

ROMANIA:

S-5221: Vama Veche village, 10 km south of Mangalia, well, July 24, 1994, many specimens (holotype and paratypes) (leg. M. Sarbu).

**LOCALITIES CITED**: **Bulgaria:**

Andreev, 2001: cited *Niphargus* sp. from Chabla Lake, v. Chabla, reg. Varna.

Karaman, G. 1922: Shabla, Tolbuhin region.

**DISTRIBUTION**: Romania, Bulgaria.

### *NIPHARGUS DOBROGICUS* Dancau, 1964

*Niphargus dobrogicus* Dancau, 1964: 397, figs. 1-3; Andreev, 1972: 62; Barnard, J.L. & Barnard, C.M., 1983: 691; Petrescu, 1996: 211.

**LOCUS TYPICUS**: Well in village Schitu, Dui Mai, Vama Veche, reg. Dobrogea, Romania (near coast of Black Sea).

**LOCALITIES CITED**:

BULGARIA**:**

Petrescu, 1996: Chabla near Black Sea;

Andreev (1972) mentioned on p. 62:" in spring and in well in village Chabla and locality Tauk Liman, NE of-Varna".

ROMANIA:

Dancau, 1964: Schitu; 2 Mai; Vama Veche;

Petrescu, 1996: Schitu; 2 Mai; Vama Veche.

**DISTRIBUTION**: Bulgaria, Romania.

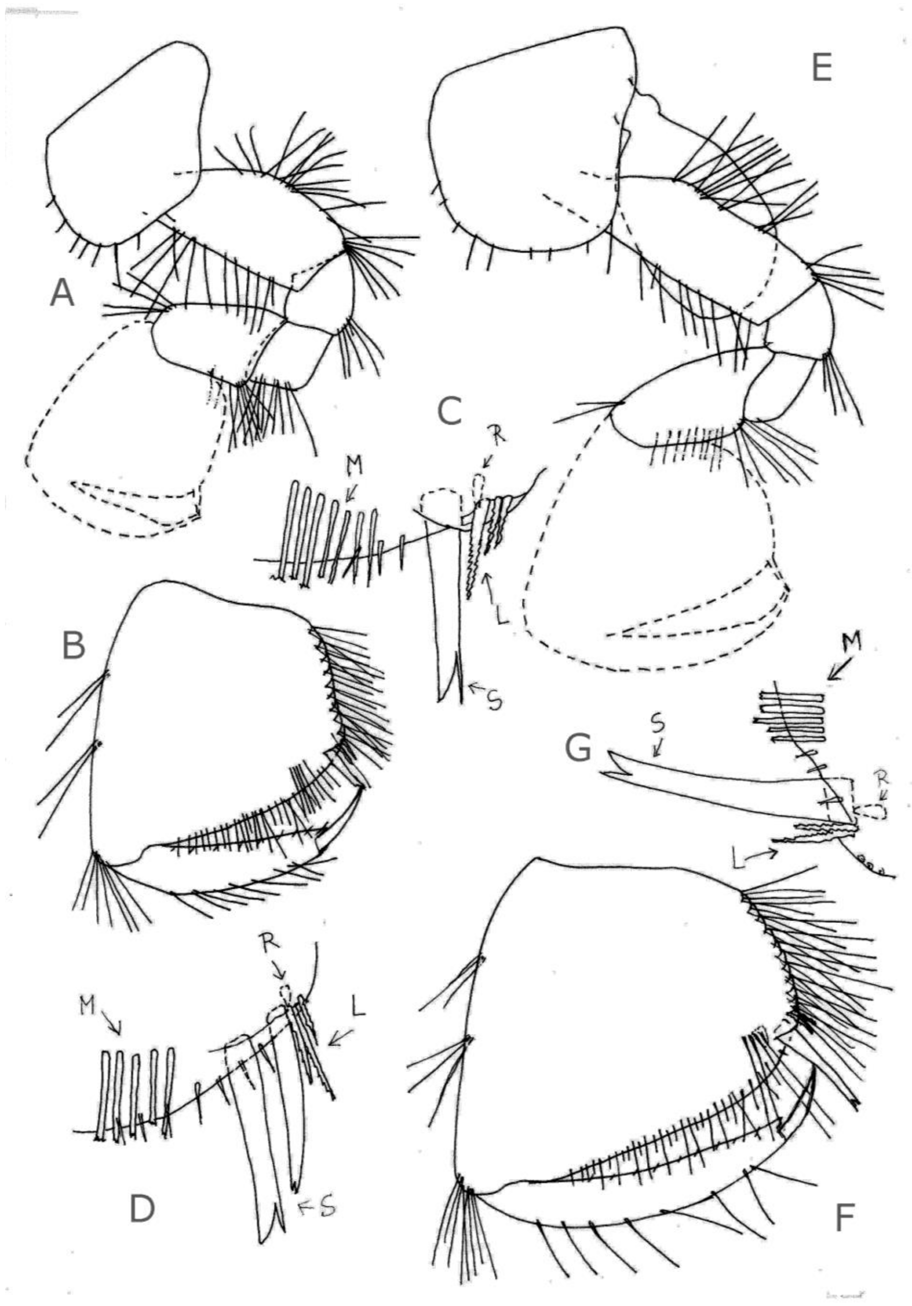


Fig. 5. *Niphargus vlkanovi burgasi*, ssp. nov., Verovnik, Burgas, male 15.5 mm (holotype): A-B= gnathopod 1, outer face; C= facial corner of left gnathopod 1 propodus (S= corner S-spine; L= lateral L-spines; M= corner facial M-setae; R= subcorner R-spine, inner face); D= facial corner S-spines of right gnathopod 1 propodus; E-F= gnathopod 2; G= facial corner of right gnathopod 2 propodus.

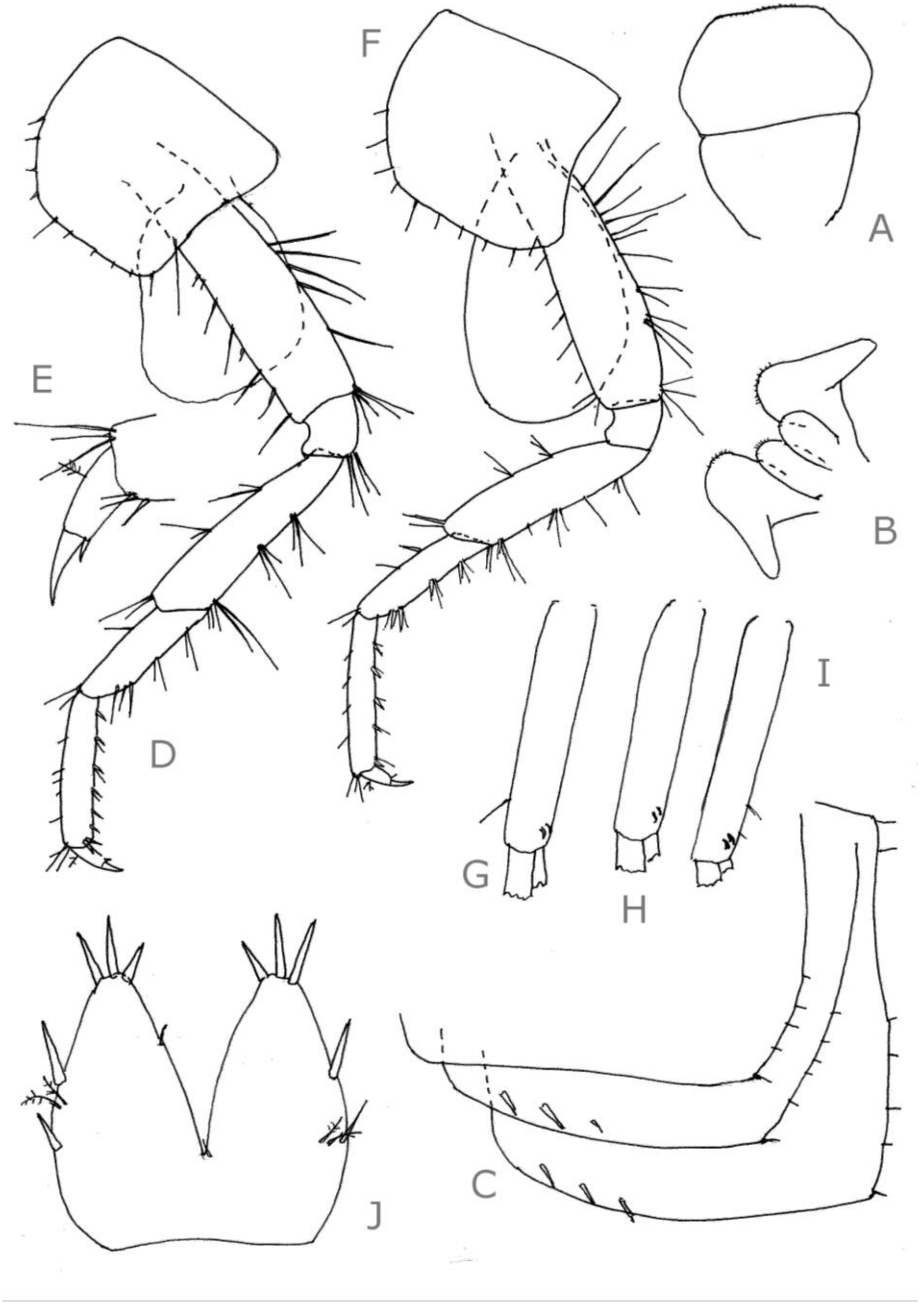


Fig. 6. *Niphargus vlkanovi burgasi*, ssp. nov., Verovnik, Burgas, male 15.5 mm (holotype): A= labrum; B= labium; C= epimeral plates 1-3; D-E= pereopod 3; F= pereopod 4; G= peduncle of pleopod 1; H= peduncle of pleopod 2; I= peduncle of pleopod 3; J= telson.

**REMARKS**

Andreev (1972) mentioned specimens bearing pleopods with 6 retinacula, uropod 1 outer ramus 1/5 shorter than inner ramus, telson with only one sensitive seta subapical on lateral margin. He considered that small differences are present because of non adult specimens in hands.

### *NIPHARGUS GEORGIEVI* S. Karaman & G. Karaman, 1959

Figs. 11-12

*Niphargus ablaskiri georgievi*, S. Karaman & G. Karaman, 1959: 148, figs. 9-16; Andreev, 1972: 64; Barnard, J.L. & Barnard, C.M., 1983: 689; G. Karaman & Ruffo, 1986: 522; Beron, 1994: 13;

*Niphargus georgievi* G. Karaman, 1973: 495.

**LOCUS TYPICUS:** S. Karaman & G. Karaman (1959) do not cited locus typicus; G. Karaman (1973a) and we selected Ourouchka peštera Cave near village Krochouna, Lovetch as locus typicus, because the most of description and figures are of specimens from this cave.

**MATERIAL EXAMINED**: BULGARIA:

Holotype Ourouchka peštera Cave, slides.

X-630= Popova peŝtera Cave near village Krochouna, 1 exp. Lovetch reg. (leg.?);

S-2414= Divitaŝka peŝtera-Cave, Lovetch (= Loveč) district, Bulgaria, 5.7.1922, 1 male (leg.?).

BU-5= Cave near Mussina, district Veliko Tarnovo, 12.10.1979, 8 exp. (leg. P. Beron, A. Popov, & S. Andreev);

IP-15A= Cave Malkata vodna, village Micre, dept. Lovetch, subterranean torrent, 19.1.1992, 2 exp. damaged (leg? ) cf. *georgievi.*

**LOCALITIES CITED:** BULGARIA:

S. Karaman & G. Karaman, 1959: 0urouchka peštera Cave and Popova peštera Cave near village Krochouna, Lovetch.

Andreev, 1972 mentioned existence of 2 localities described by S. & G. Karaman (but without name of localities).

Beron, 1994: Zmejovi Dupki (Gb 5); Vodnata Velichovska peštera Cave (Gb 9).

**DISTRIBUTION**: Bulgaria, endemic.

**REMARKS**.

This species was described and figured by S. & G. Karaman (1959) from 2 caves of Lowetch region (Popova and Ourouchka Caves) based on males and females up to 14 mm long. Andreev (1972) mentioned that this species is known from one larger region with some other localities (but without names). He agrees with original known description of this species and cited lobes of telson with 1-3 lateral spines, 4-6 apical spines, and the number of retinacula on pleopods 1-3 (4-5-6 to 5-6-7). Later this species was neither redescribed nor mentioned some supplementary morphological data, and we mentioned here some additional data.

Epimeral plates distinctly pointed, plate 3 rather produced, plates 2 and 3 with various number of subventral spines (Fig. 11E).

Urosomal segment 1 with one seta on each dorsolateral side; urosomal segment 2 on each side with 4-5, sometimes to 7 spines; urosomal segment 3 naked. This curiosity of urosomal spinulation was observed already by S. & G. Karaman (1959). Urosomal segment 1 on ventroposterior corner with one spine near basis of uropod 1-peduncle.

Mandibular palpus article 1 naked, article 2 with 15 setae; article 3 nearly as long as article 2, subfalciform, with nearly 30 marginal D-setae and 4-6 distal E-setae, on outer face with 7 facial A-setae, on inner face with 6 facial B-setae (2+2+2) (Fig. 11A).

Maxilla 1 inner plate with 1-2 setae (Ourouchka Cave, Popova Cave, Divitaška Cave) or 3 setae [(Messina, Veliko Tarnovo (Fig. 11H)], outer plate with 7 spines (6 with one lateral tooth, inner spine with 3 lateral teeth), palpus nearly reaching distal tip of outer plate spines (Fig. 11B), bearing usually 6 distal and 2 distomesial setae.

Maxilliped: inner plate with 5-6 distal spines mixed with single setae, palpus usually with 2 mesial setae near basis of the nail (Fig. 11C).

We measured coxal plates 1-4 of Divitaška Cave, male 9 mm. Coxa 1 and 4 are scarcely longer than broad (Fig. 12A, D), coxae 2 and 3 distinctly longer than broad (Fig. 12B, C); coxa 4 with very shallow ventroposterior lobe (Fig. 12D), all coxae scarcely setose.

Gnathopods 1-2: article 3 provided with one distoposterior bunch of setae; article 5 rather shorter than propodus. Gnathopod 1 propodus much smaller than that of gnathopod 2, trapezoid, rather longer than broad, along posterior margin with nearly 10 transverse rows of setae (Fig. 12A); palm inclined poorly less than half of propodus-length, convex, defined on outer face by corner S-spine accompanied laterally by 3 serrate L-spines and corner facial 3 M-setae, on inner face by one subcorner R-spine. Dactylus at outer margin with one median seta, along inner (mesial) margin with several short setae.

Gnathopod 2 propodus trapezoid, nearly as long as broad, along posterior margin with nearly 12 transverse rows of setae (Fig.12B): palm inclined nearly half of propodus-length, convex, defined on outer face 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. Dactylus with one median seta at outer margin and several short setae at inner margin.

Pereopods 3-4 dactylus moderately slender, with one spine at inner margin, nail shorter than pedestal.

Pereopods 5-7 moderately long, pereopod 5 shorter than pereopods 6-7, all with articles 3-6 bearing groups of spines mixed with setae nearly as long as spines. In female pereopod 7 article 2 only slightly shorter than article 6 (ratio: 70:80); article 2 rather narrowed, longer than broad (ratio: 70:42), posterior margin poorly convex, with nearly 13 setae (Fig. 11D), ventroposterior lobe absent. Dactylus moderately strong, with one spine at inner margin and one median seta at outer margin, nail shorter than pedestal (Fig. 11D).*Niphargus kragujevensis femineus* G. Karaman, 2024b: 15, figs. 3-8.

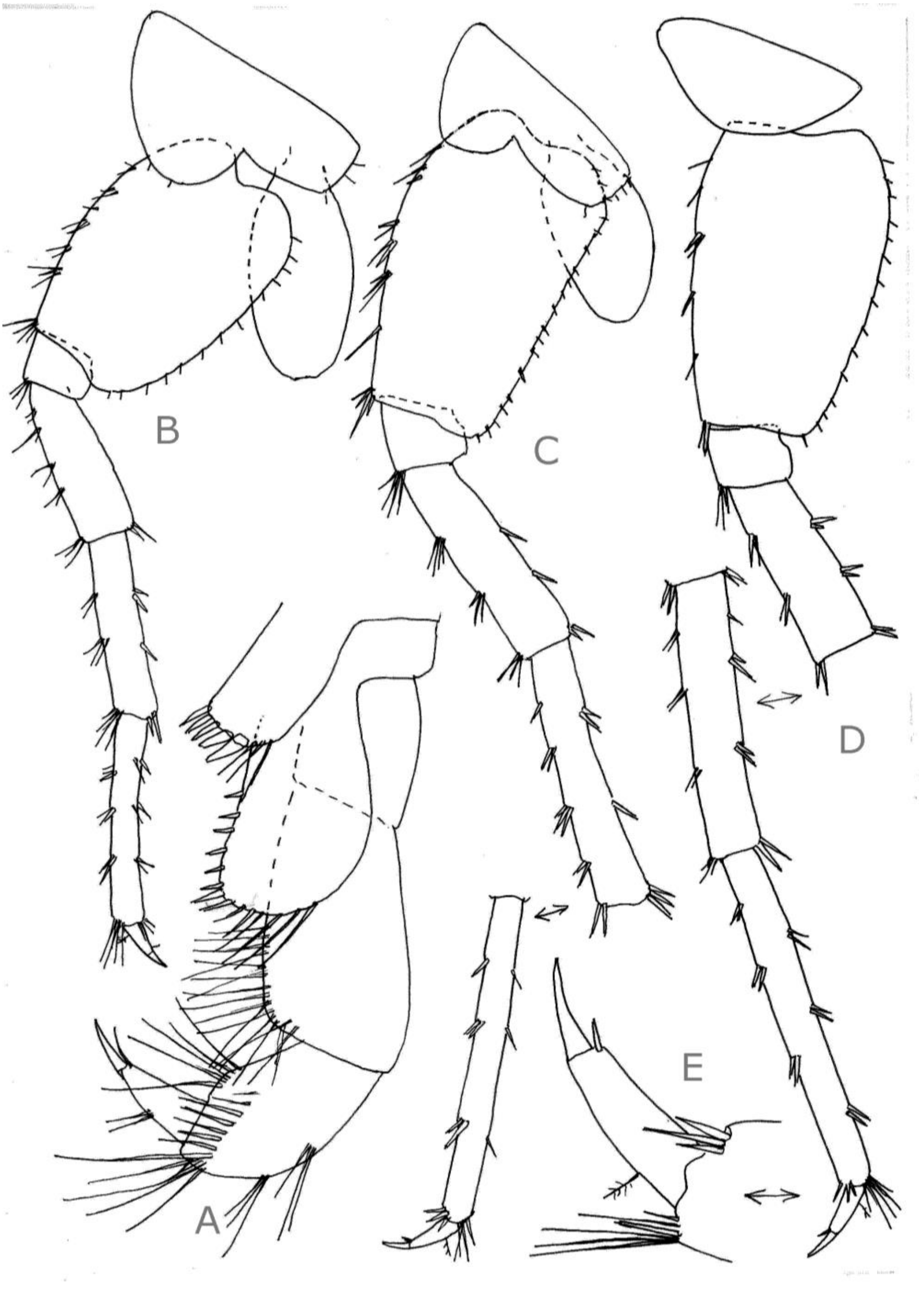


Fig. 7. *Niphargus vlkanovi burgasi*, ssp. nov., Verovnik, Burgas, male 15.5 mm (holotype): A= maxilliped; B= pereopod 5; C= pereopod 6; D-E= pereopod 7.

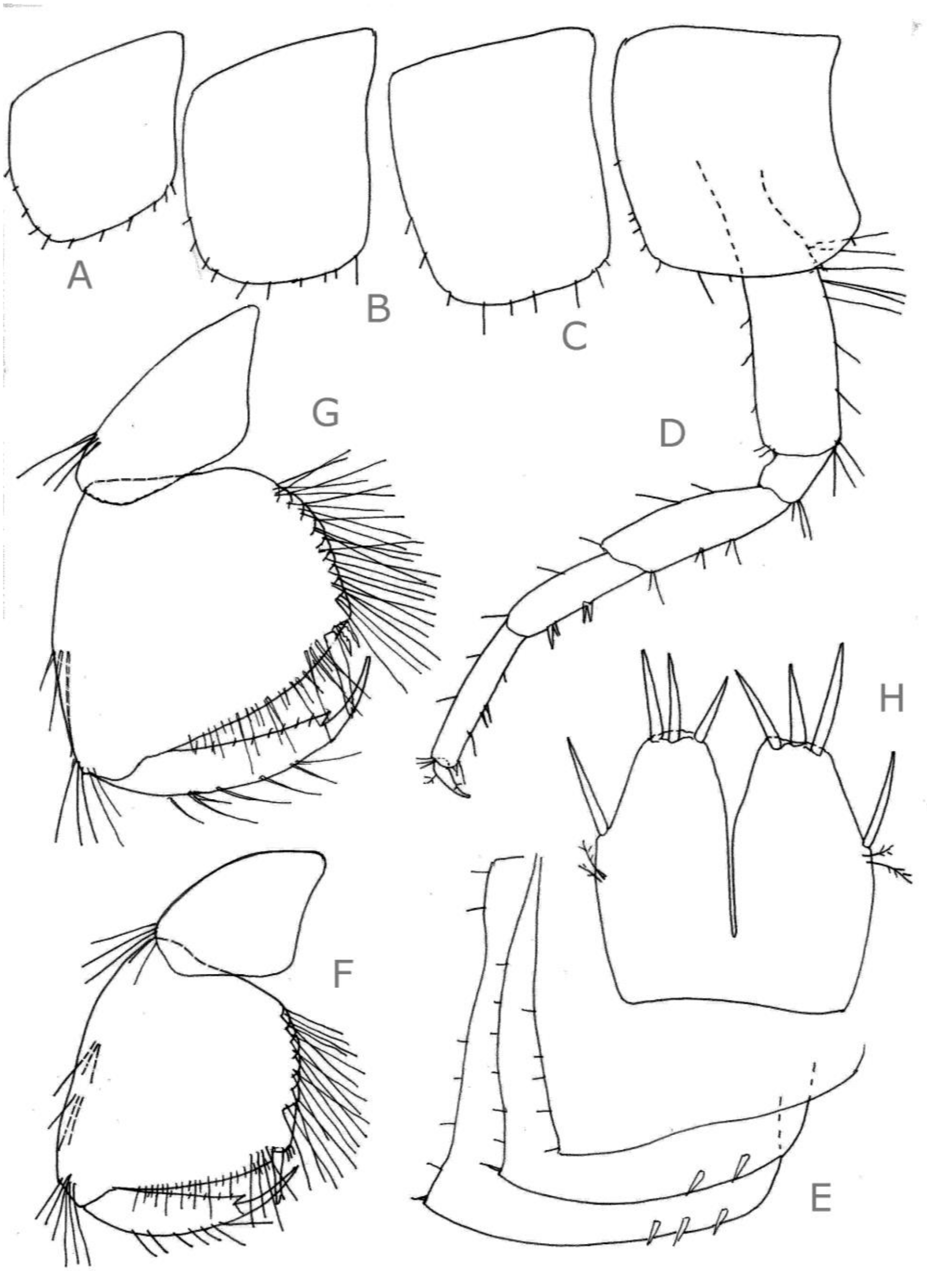


Fig. 8. *Niphargus vlkanovi burgasi*, ssp. nov., Verovnik, Burgas, female 12.0 mm (paratype): A= coxa 1; B= coxa 2; C= coxa 3; D= coxa 4 and pereopod 4; E= epimeral plates 1-3; F= gnathopod 1; G= gnathopod 2; H= telson.

Pleopods with elevated number of retinacula, usually 4-6. Peduncle of pleopods 1-3 scarcely setose, bearing several single short setae only or naked.

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae. Rami of equal length, with several lateral spines and 4-5 distal short spines. Uropod 2 with subequal rami bearing single lateral and 4-5 distal short spines.

Uropod 3 long in male, with short peduncle and short, scale-like, inner lobe bearing distal spine (Fig. 11G). Outer ramus 2-articulated, in adult specimens articles are of equal length: first article along outer margin with several single short spines, along inner margin with row of single short spines mixed with single long plumose setae; second article along both margins with single of paired short setae, at top with several simple setae. Subadult males and females are with second article of outer ramus more or less shorter than first one.

Telson broader than long, gapping, not tapering distally, incised to the 3/4 of telson-length; each lobe with 4 distal and 1-2 outer marginal strong spines, one pair of short plumose setae siting near the middle of outer margin (fig. 12E).

Coxal gills ovoid, not elongated (Fig. 12B, C, D).

**In female (9.0 mm, Divitaška peštera)** uropod 3 shorter and broader than in male (Fig. 11F). Peduncle short, poorly longer than broad, with single distal spines; inner ramus short, scale-like, with distal spine and seta; outer ramus 2-articulated, first article along outer margin with 4 groups of short spines, along inner (mesial) margin with several short spines mixed with single plumose setae; second article rather shorter than half of first article, along both margins and tip with single or groups of short simple setae.

**VARIABILITY**. The number of spines on telson is rather variable, usually on each lobe 3-4 distal spines and 1-2 lateral spines, but no facial spines; distal tip of lobes is more or less broad, not narrowed like these in *N. bureschi*. Inner plate of maxilla 1 with 1-2 setae, possible also 3. Inner plate of maxilliped is provided with 3-6 distal spines in various localities.

The specimens from Malkata vodna Cave (juv. 4.8 mm) seems belong to this species, having acute epimeral plates, elevated number of retinacula (3), one outer marginal seta on gnathopod dactylus, and 3-4 spines on each side of urosomal segment 2.

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## *NIPHARGUS JOVANOVICI* S. Karaman, 1931
(Shortened synonymy).

*Niphargus jovanovici* S. Karaman, 1931: 93, figs. 1-2; G. Karaman, 1980: 17; Pesce & Maggi, 1983: 58; Beron, 2015: 35;

*Niphargus jovanovici jovanovici* Schellenberg, 1935: 206 (key); S. Karaman, 1943: 173, 207, pl. III, figs. 43-62; Dancau, 1963: 473 (key);

*Niphargus* (*Jovaniphargus*) *jovanovici jovanovici* S. Karaman, 1960: 86, fig. 5; Dancau, 1963: 473 (key); Sket, 1972: 10, fig. 107;

nec *Niphargus jovanovici jovanovici* Dobreanu, Manolache & Puscariu, 1951: 579, figs. 1-2 (= *N. serbicus* S. Karaman, 1960*).*

**LOCUS TYPICUS**: Skoplje, Northern Macedonia.

**LOCALITIES CITED:** BULGARIA:

Beron, 2015: **page: 227**: Mt 13. Shokyovets (Shyokovets) cave near Cherkaski Village. Length 144 m. Deniv. 6 m., underground stream; **page 230**: Mt 35. Sushitsa Cave near Studeno Buche Village. Length 31 m.

Pesce & Maggi, 1983A: "Grecia settentrionale and Isole Ioniche".

**DISTRIBUTION**: Northern Macedonia, Greece, Serbia, Bulgaria.

## *NIPHARGUS KRAGUJEVENSIS FEMINEUS* G. Karaman, 2024b

*Niphargus kragujevensis femineus* G. Karaman, 2024b: 15, figs. 3-8.

**LOCUS TYPICUS**: Ledenicata Cave near vil. Gela (Smoljan region, Rhodopes Mts.), Bulgaria.

**MATERIAL EXAMINED**:

Ledenicata Cave near vil. Gela (Smoljan region, Rhodopes Mts.), Bulgaria. 3.11.1969, 2 exp. (leg. A. Popov).

**LOCALITIES CITED**: BULGARIA.

Karaman, G. 2024: locus typicus.

**DISTRIBUTION**: Bulgaria, endemic.

**REMARKS**: see key.

## *NIPHARGUS KRAGUJEVENSIS REMUS* G. Karaman, 1992

*Niphargus kragujevensis remus* G. Karaman, 1992: 20, figs. V-IX; G. Karaman, 1994: 231; G. Karaman, 1995: 324; G. Karaman, 1997: 350; G. Karaman, 1998b: 236; G. Karaman, 1999: 168; G. Karaman, 2011: 150; G. Karaman, 2024b: 10, figs. 1-2.

**LOCUS TYPICUS**.: Fountain above the village Prekonoga, Svrljig Mt., Serbia.

**MATERIAL EXAMINED**: BULGARIA:

IP-7= Gallery artificial "Urvitch" near Sofia, valley of Iskar River, 23.4.1993, 3 exp. (leg.?).

S-7435= Sozopol, in roots (E. of Burgas, coast of Black Sea), 17.12.1963, 3 exp. [leg. Lj. Cvetkov).

**LOCALITIES CITED:** BULGARIA:

Karaman, G. 2024: IP-7= Gallery artificial "Urvitch" near Sofia, valley of Iskar River.

**DISTRIBUTION**: Bulgaria, Serbia.

**REMARKS**. The specimens from Bulgaria agree with description of ssp. ***r**emus* from Serbia.

### *NIPHARGUS MELTICENSIS* Dancau & Andreev, 1973

*Niphargus kochianus melticensis* Dancau & Andreev, 1973; 135, figs. 1-4; Barnard, J.L. & Barnard, C.M., 1983: 692.
*Niphargus melticensis* G. Karaman & Ruffo, 1986: 528.

**LOCUS TYPICUS**: Well in Sokolovo, Lovetch district, Bulgaria.
**LOCALITIES CITED**: locus typicus only.
**DISTRIBUTION**: Bulgaria, endemic.

### *NIPHARGUS MERIDIONALIS* Dobreanu & Manolache, 1942

*Niphargus carpathicus meridionalis* Dobreanu & Manolache, 1942: 301, figs. 13. 15, Barnard, J.L. & Barnard, C.M., 1983: 690; Petrescu, 1996: 208, figs. 18-22: G. Karaman, 2023: 15.
Ceatalar (= Batovo), Bulgaria.

**LOCUS TYPICUS**: Ceatalar (= Batovo), NE Bulgaria.
**LOCALITIES CITED**:
Dobreanu & Manolache, 1942: Ceatalar (Caliacra) (= Batovo), Bulgaria;
Petrescu, 1996: Batovo (Bulgaria); Furnica (Romania).
G. Karaman, 2023: Ceatalar (= Batovo), Bulgaria.

**DISTRIBUTION**: Bulgaria, Romania.

**REMARKS**.

Dobreanu & Manolache described (1942) *Niphargus carpathicus meridionalis*, ssp. nov. from Ceatalar (Caliacra) (= Batovo). As this region after the Second World War belongs to Bulgaria, Carausu, Dobreanu & Manolache (1955) from Romania omitted this taxon in their book of fauna of Amphipoda from Romania.

Dobreanu & Manolache (1942) in his rather short and incomplete description of *N. c. meridionalis* mentioned that gnathopods are mainly like these of *N. romanicus* Dobreanu & Manolache, 1942. But *N. romanicus* is with dactylus of gnathopods with one median seta at outer margin, what was mentioned already by G. Karaman (2023). Uropod 3 is not described, as well as the number of retinacula [“retinaculum est formé de 3 crochets”].

Petrescu (1996) redescribed this taxon from Batovo Valley and mentioned it for Furnica in SE Romania, not far from Bulgaria border, nearly 60 km N. of Batovo. Dactylus of gnathopod 1 is figured with 5 setae, that of gnathopod 2 with 2 setae; pleopods are with 2 retinacula.

This taxon is quite different from *Niphargus carpathicus*-complex of taxa, based on presence of several setae on outer margin of gnathopods 1-2 propodus, and represent a distinct species, *Niphargus meridionalis* Dobreanu & Manolache, 1942, with type-locality: Ceatalar (= Batovo), Bulgaria.

## ***NIPHARGUS PECARENSIS*** S. Karaman & G. Karaman, 1959

*Niphargus tauri pecarensis*, n. ssp., S. Karaman & G. Karaman, 1959, 143, figs. 1-8; Andreev, 1972: 62; Barnard, J.L. & Barnard, C.M., 1983: 696; G. Karaman & Ruffo, 1986: 533; Beron, 1994: 13, 486; G. Karaman, 2013: 220;

*Niphargus pecarensis pecarensis*, G. Karaman, 1998a, 116, figs. 1-4; G. Karaman, 1999: 168.

**LOCUS TYPICUS**: Cave ‚Pečara dupka" near town Belogradčik in NW Bulgaria.

**LOCALITIES CITED**: BULGARIA

S. Karaman & G. Karaman, 1959: locus typicus.

Andreev, 1972 cited this species for cave "Goliami Petch" near village Varbovo, mixed with *N. bureschi*, in the subterranean running waters.

Beron, 1994: Bulgaria, stygobiont: Haidushkata propast (Vd 5); Jame II (Vd 28); Right suhi petch (Vd 13); Prelaz (Vd 21);

SERBIA:

G. Karaman, 1999; Cave near Gabrovica Village by Kalna, Serbia.

**DISTRIBUTION:** Bulgaria, Serbia.

## **?*NIPHARGUS PUTEANUS* Koch, C.L., 1836**

*Gammarus puteanus* Koch, in Panzer 1936: V. 5, n. 2.

*Niphargus puteanus* (? Koch, 1836) Fage, 1926: 6; Beron et al., 2011: 591 [updated 2017].

**LOCUST TYPICUS:** Weichselmühle near Regensburg, Germany**.**

**LOCALITIES CITED**:

Fage 1926: Grotte de Progled, Ċepelare district, Rhodopes Mts., 1000 m.

Beron, Petrov & Stoev, 2011: Sbirkovata peshtera (Sm 4) cave near Progled Village. Alt. 1430 m. Temp. 6 C.(reg. Smolyan) [from the same cave he mentioned also *N. cepelarensis*].

**DISTRIBUTION**: Central Europe, borders of distribution areal is not clear.

**REMARKS**. Specimens mentioned for Bulgaria probably belong to some other species.

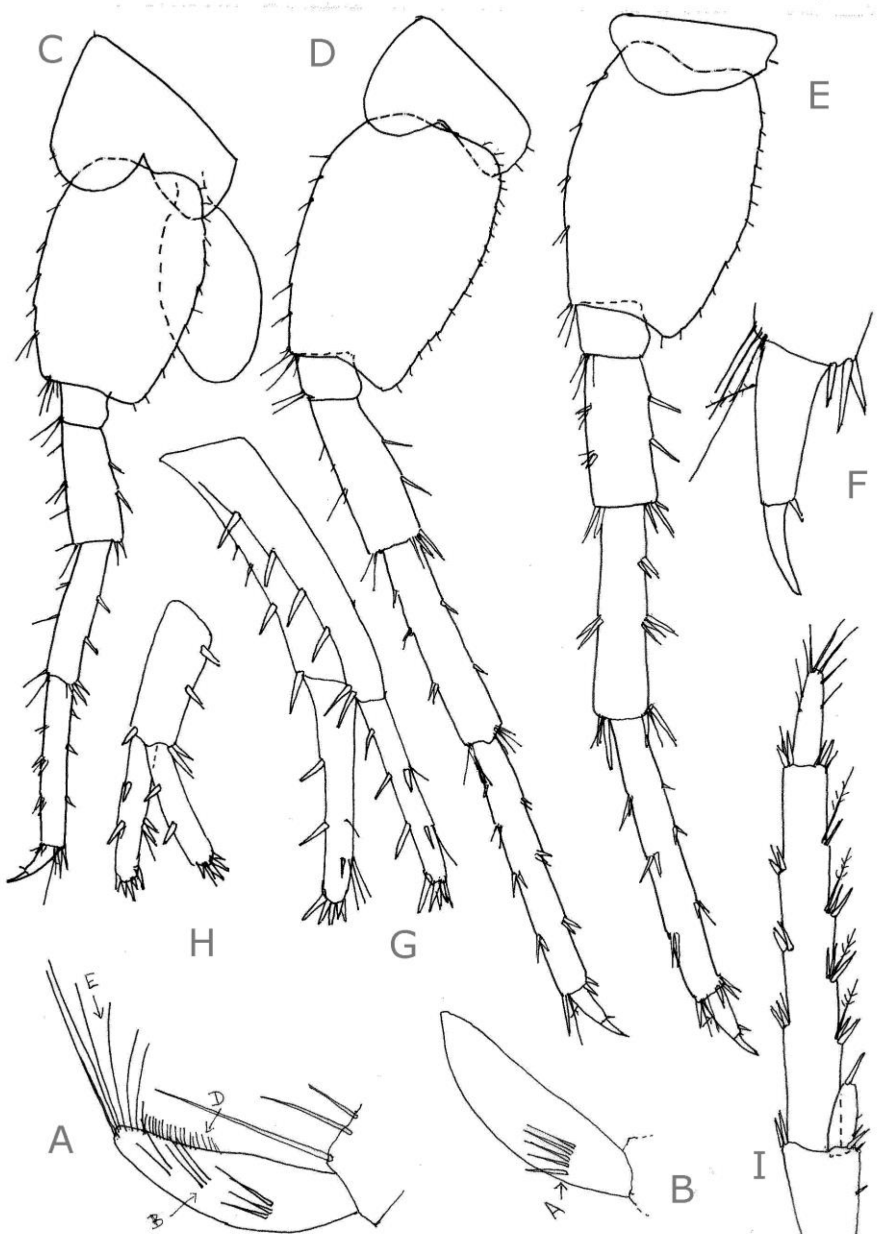


Fig. 9. *Niphargus vlkanovi burgasi*, ssp. nov., Verovnik, Burgas, female 12.0 mm (paratype): A= mandibular palpus, inner face (B=facial B-setae); D= marginal D-setae; E= distal E-setae); B= mandibular palpus, outer face (A= facial A-setae); C= pereopod 5; D= pereopod 6; E-F= pereopod 7; G= uropod 1; H= uropod 2; I= uropod 3.

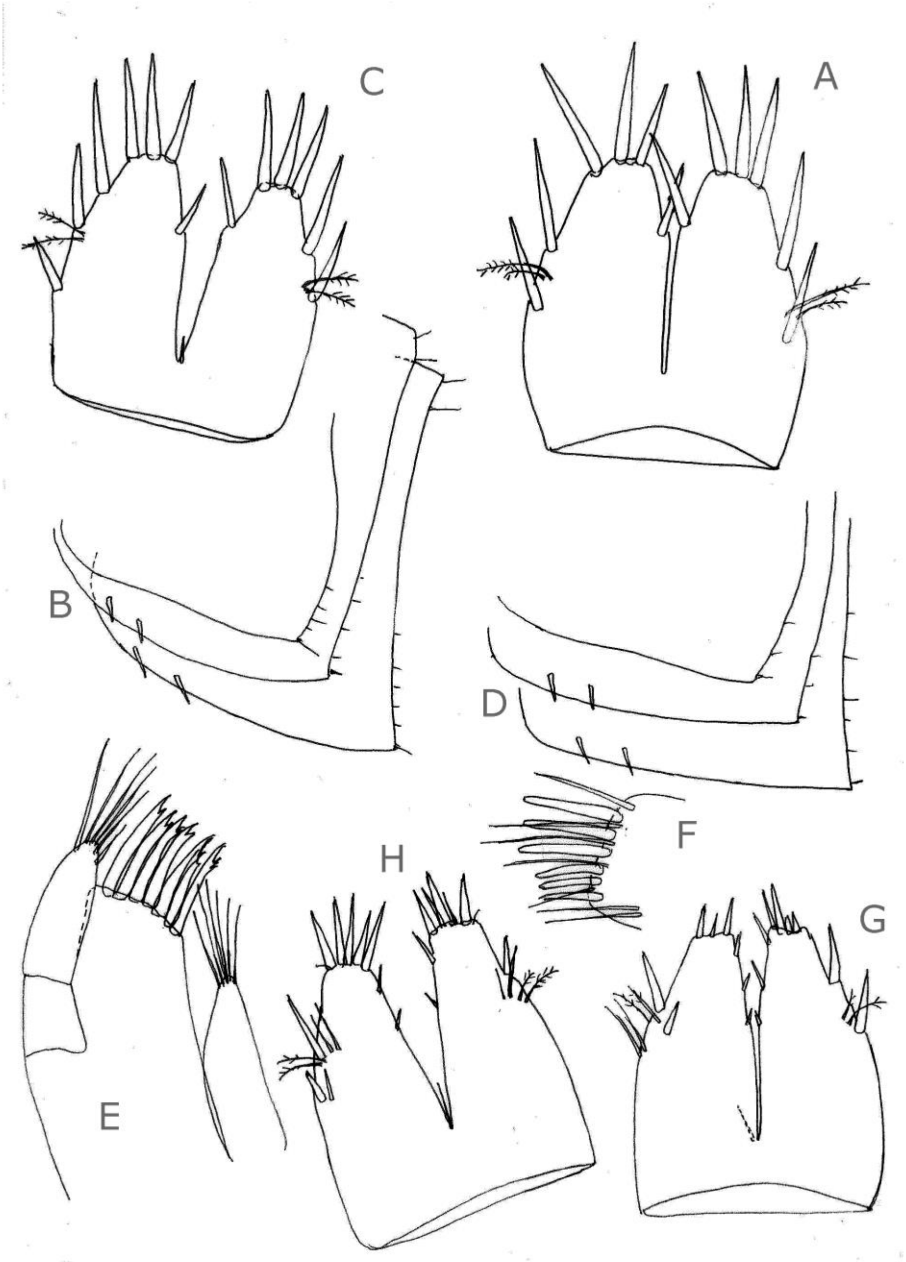


Fig. 10. *Niphargus vlkanovi burgasi*, ssp. nov., Petrova niva, Stoilovo: female 11.0 mm: A= telson; B=, epimeral plates 1-3. C= female 11.1 mm, telson' **Male 11.6 mm**: D= epimeral plates 1-3.
***Niphargus bureschi*** **Fage 1926**, Temnata Dupka Cave, male 19.1 mm: E= maxilla 1; F= maxilliped, inner plate; G= telson; H= **female 15.5 mm**. telson.

## *NIPHARGUS TOPLICENSIS* Andreev, 1966

*Niphargus toplicensis* Andreev, 1966: 483, pls. 53, 54; Andreev, 1972: 64; G. Karaman & Ruffo, 1986: 533; Barnard, J.L. & Barnard, C.M., 1983: 696.

**LOCUS TYPICUS**: spring "Toplitzata" near village Mussomischta, Valley of Mesta River (reg. Goce Deltchev), SW. Bulgaria.

**MATERIAL EXAMINED**:

EE-38: spring Toplika near Goce Delchev, 21.5.1962, 12 exp. (leg. V.B. Georgiev). This is topotypic material, of the same sample as holotype.

**LOCALITIES CITED**: BULGARIA

Andreev, 1966: spring "Toplitzata" near village Mussomischta (reg. Goce Deltchev);

Andreev, 1972: karstic spring of the river Mesta (SE Bulgaria); also second spring in the same region (without name).

**DISTRIBUTION**: Bulgaria, endemic.

**REMARKS**. Male 9 mm in hands: urosomal segment 1 with 1 strong seta on each dorsolateral side, urosomal segment 2 with 1 spine on each dorsolateral side, urosomal segment 3 naked.

Maxilla 1 inner plate with 3 setae, palpus distinctly reaching basis of outer plate spines. Gnathopods 1-2 article 3 with one distoposterior bunch of setae; palm of propodus on outer face defined by corner strong S-spine accompanied laterally by 2 serrate short L-spines and facial corner 3 M-setae, on inner face by one subcorner R-spine. Pleopods with 2 retinacula, peduncle of pleopods almost naked, with 0-3 short setae only. Telson without lateral spines, lobes lobes with 1 facial spine and nearly 3-4 distal spines.

## *NIPHARGUS VLKANOVI VLKANOVI* S. Karaman & G. Karaman, 1959

Figs.: 1-3

*Niphargus pancici vlkanovi*, S. Karaman & G. Karaman, 1959, 155, figs. 21-29; Andreev, 1966: 285; Andreev, 1972: 62; Barnard, J.L. & Barnard, C.M., 1983: 694; G. Karaman & Ruffo, 1986: 529; Beron, 1994: 13, 486.

? *Niphargus tatrensis* Schäferna, 1922: 89.

**LOCUS TYPICUS**: Cave near Živata voda (=Zhivata voda), S. part of Vitoscha Mt., 1100 m. asl., Sofia region, Bulgaria.

**MATERIAL EXAMINED**: BULGARIA

X-635= Cave near Živata Voda, Sofia reg., 5.2.1925 (leg. Buresch), slides paratype and holotype).

**LOCALITIES CITED:** All authors cited type locality only.

Schäferna 1922: Vitosha Mt. 800 m asl., Bulgaria [sub N. tatrensis) probably].

**DISTRIBUTION**: Bulgaria, endemic.

**VARIABILITY:** Holotype and paratype were at disposition for study only.

**REMARKS**. This taxon was described and partially figured by S. Karaman & G. Karaman (1959) based on scarce material. For this reason, I have revised the holotype and paratype on slides to complete description and made some additional figures of both sexes.

**Male (holotype on slide) 12.0 mm:** Metasomal segments with 4-6 dorsoposterior marginal setae (Fig. 2F); urosomal segment 1 on each dorsolateral side with 1 seta, urosomal segment 2 on each dorsolateral side with 1 seta, urosomal segment 3 naked. Urosomal segment 1 at ventroposterior corner with one spine near basis of uropod 1 (Fig. 1E).

Epimeral plates 1-3 subrounded (Fig. 2F), plates 2-3 with 1-3 ventral spines.

Head with short rostrum and subrounded lateral cephalic lobes. Antenna 1 reaching nearly half of body-length, peduncular articles 1-3 progressively shorter, scarcely setose; last peduncular article short; main flagellum consisting of 21 articles (many of them with one short aesthetasc). Accessory flagellum 2-articulated, short, reaching nearly 2/3 of last peduncular article (Fig. 2A).

Antenna 2 moderately slender, peduncular article 5 poorly shorter than 4, both with several groups of setae (the longest setae exceeding diameter of article itself); flagellum longer than last peduncular article, consisting of 9 articles. Antennal gland corner short.

Mouthparts well developed. Mandible palpus article 1 naked, article 2 with 10 setae, article 3 with D, E, A and B-setae.

Maxilla 1 inner plate with one seta, outer plate with 7 spines (6 spines with one lateral tooth, inner spine with 3 lateral teeth), palpus not reaching distal tip of outer plate-spines, provided with 6 setae (Fig. 1A).

Maxilliped: inner plate short, with 3 distal spines mixed with single setae (Fig. 1B); palpus article 4 at inner (mesial) margin with one seta near basis of the nail.

Coxae relatively short. Coxa 1 much broader than long (ratio: 56 37) (Fig. 2B); coxa 2 broader than long (ratio: 69:48) (Fig. 2C); coxa 3 rather broader than long (ratio: 70:57) (Fig. 2D); coxa 4 without distinct ventroposterior lobe, broader than long (ratio: 74:55) (Fig. 2E).

Gnathopods 1-2 poorly larger than corresponding coxae. Gnathopod 1 only slightly smaller than gnathopod 2, article 3 with one distoposterior bunch of setae; article 5 rather shorter than propodus (ratio: 58:75), with distoanterior bunch of setae. Propodus trapezoid, rather longer than broad (ratio: 79:75), along posterior margin with 5 transverse rows of setae. Palm only slightly convex, inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 serrate L-spines and 5 corner facial M-setae (Fig. 2G), on inner face by subcorner R-spine. Dactylus along outer margin with 7-8 single of paired median setae, along inner (mesial) margin with row of short setae.

Gnathopod 2: article 3 with one distoposterior bunch of setae; article 5 rather shorter than propodus (ratio: 68:86), with distoanterior bunch of setae (Fig. 2H). Propodus trapezoid, nearly as long as broad (ratio: 86:90), along posterior margin with 6 transverse rows of setae. Palm poorly convex, inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 L-spines and 5 corner facial M-setae, on inner face by subcorner R-spine, Dactylus with row of 6-7 single or paired median setae along outer margin, and several short setae along inner margin (Fig. 2H).

Pereopods 3-4 moderately slender, scarcely setose. Pereopod 3: articles 4-7 of different length (ratio: 80:53:60:26), dactylus with slender spine at inner margin near basis of the nail, nail poorly shorter than pedestal. Pereopod 4 similar to pereopod 3, almost as long as pereopod 3.

Pereopods 5-7 scarcely spinose. Pereopod 5 shorter than pereopods 6 and 7, with article 2 rather longer than broad, with 6 setae along posterior margin, ventroposterior lobe absent.

Pereopod 6: article 2 rather longer than that in pereopod 5, with 8-9 posterior marginal setae and without ventroposterior lobe.

Pereopod 7: article 2 remarkably longer than broad (ratio: 74:40), along anterior poorly convex margin with 6 single spine-like setae, along posterior poorly convex margin with 11 short setae, ventroposterior lobe absent (Fig. 1C); articles 4-7 of different length (ratio: 40:60:72:28); articles 4-6 along both margins with single or pair of spines shorter than diameter of articles themselves. Article 6 almost as long as article 2 (ratio: 72:74). Dactylus with spine at inner margin and one median seta at outer margin (Fig. 1D), nail rather shorter than pedestal.

Pleopods 1-3 with 2 retinacula, peduncles almost naked.

Uropod 1: peduncle shorter than inner ramus, with dorsoexternal row of spines, dorsointernal row naked (except distal spine-like seta); inner ramus long, along ventrolateral margin wit 6 bunches of setae, along dorsal margin with row of short setae, distal tip with 4 short spines (Fig. 1E); outer ramus reaching almost half of inner ramus-length, with 3 groups of lateral short spines and setae and with 4 distal short spines.

Uropod 2: inner ramus poorly longer than outer one, both rami with single lateral and 4 distal short spines (Fig. 1F).

Uropod 3 very long; peduncle remarkably longer than broad (ratio: 52:20), with row of very short lateral setae and distal spines; inner ramus elongated, nearly as long as peduncle, with short simple marginal setae and distal bunch of simple setae; outer ramus 2-articulated, long, narrow, first article along inner margin with row of short simple setae, along outer margin with 5 groups of very short spines (Fig.1G); second article almost as long as first one, with 3 lateral and one distal bunch of short simple setae.

Telson almost as long as broad (ratio: 78:83), incised over 2/3 of telson-length; each lobe with 4 distal and one outer marginal spine; a pair of short plumose setae is attached near the middle of outer margin of lobes (Fig. 1H).

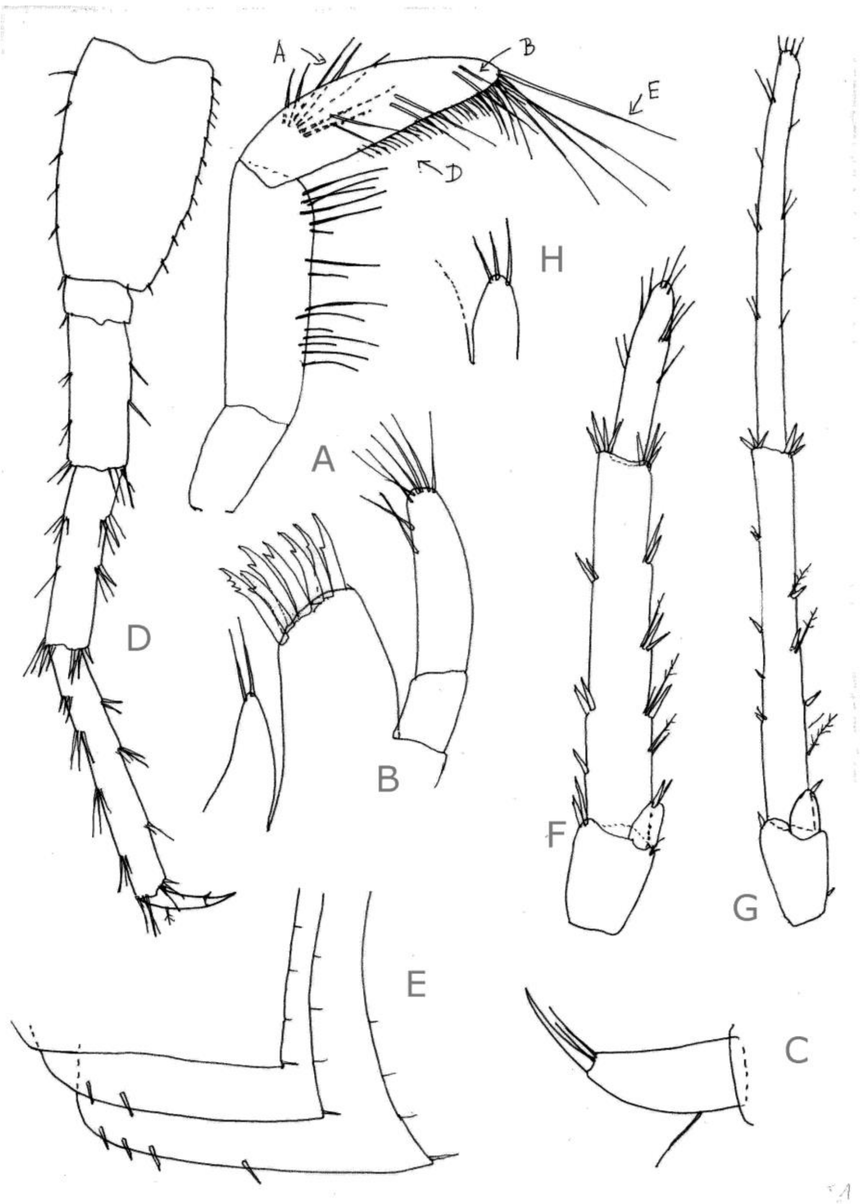


Fig. 11. *Niphargus georgievi* S. Karaman & G. Karaman, 1959, **Popova peštera Cave** near Krochouna, Lovetch Mt., female 14 mm: A= mandibular palpus, inner face (B= facial B-setae; D= marginal D-setae; E= distal E-setae; A= facial A-setae on outer face); B= maxilla 1; C= distal palpus article of maxilliped; D= pereopod 7. female 11.0 mm; E= epimeral plates, female 11.0 mm. **Divitaška peštera Cave near Krochouna**: female 9.0 mm. F= uropod 3. **Cave near Messina vil., Veliko Tarnovo reg.**, male 12.0 mm: G= uropod 3; H= maxilla 1 inner plate.

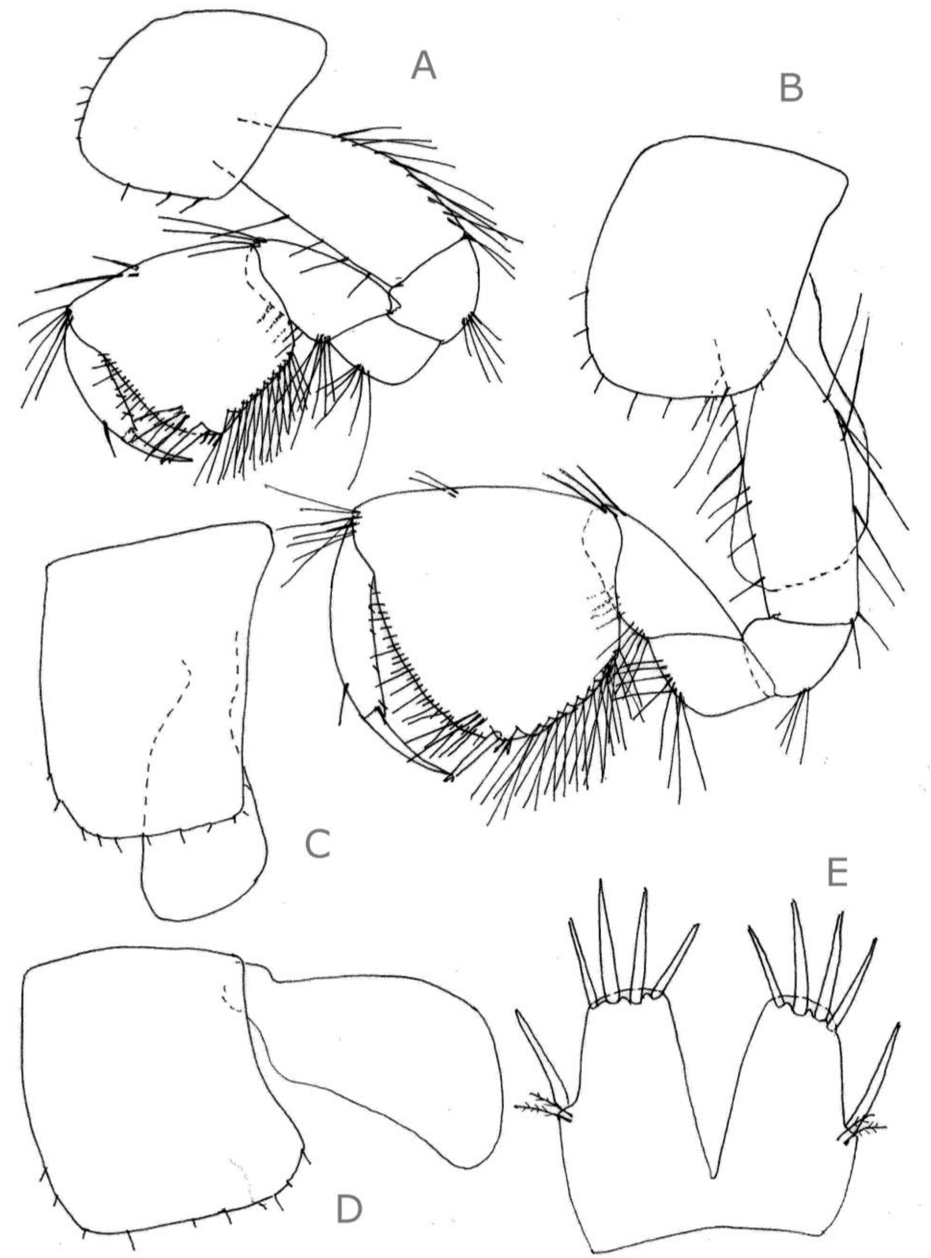


Fig. 12. *Niphargus georgievi* S. Karaman & G. Karaman 1959: Divitaška peštera Cave near Lowetch, male 9.0 mm: A= gnathopod 1, outer face; B= gnathopod 2, outer face; C= coxa 3; D= coxa 4; E= telson

Coxal gills ovoid, relatively short, coxae 2 and 4 rather longer than coxae 3 and 5.

**Female 7.0 mm, paratype, slide**: Body similar to that in male, but coxae are slightly longer. Urosomal segment 1 with one seta, urosomal segment 2 with one spine on each dorsolateral side, urosomal segment 3 naked. Urosomal segment 1 at ventroposterior corner with one spine near basis of uropod 1 (Fig. 3D).

Epimeral plates rather more angular than these in male (Fig. 2 I), plates 2 with 2 ventral spines, epimeral plate 3 with 2-3 ventral spines.

Antenna 1 not exceeding half of body, consisting of 19 articles; peduncular articles 1-3 progressively shorter, accessory flagellum like that in male. Antenna 2 with flagellum consisting of 10 articles, antennal gland cone short.

Mouthparts like these in male, but maxilla 1 inner plate with 1-2 setae.

Gnathopod 1 only rather smaller than gnathopod 2; article 3 with one distoposterior bunch of setae; article 5 rather shorter than propodus (ratio: 60:80), with one distoanterior bunch of setae. Propodus trapezoid, nearly as long as broad, along posterior margin with 5 transverse rows of setae. Palm convex, inclined nearly to the half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 L-spines and 3 corner facial M-setae, on inner face by subcorner R-spine (Fig. 3A); dactylus along outer margin with 5 median setae, along inner margin with several short setae.

Gnathopod 2: article 3 with one distoposterior bunch of setae; article 5 slightly shorter than propodus (ratio: 78:89), with distoanterior bunch of setae. Propodus trapezoid, nearly as long as broad, along posterior margin with 6 transverse rows of setae. Palm slightly convex, inclined nearly to the half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 L-spines and 3 corner facial M-setae (Fig. 3B). Dactylus along outer margin with 4-5 median setae, along inner margin with row of short setae.

Pereopods 3-4 like these in male. Pereopods 5-6 like these in male.

Pereopod 7 with article 2 longer than broad (ratio: 78:46), along anterior margin with 5 single spine-like setae and distal group of strong setae, ventroposterior lobe not marked (Fig. 3C). Articles 4-7 of different length (ratio: 41:45:74:35), articles 4-6 with single or groups of short spines along both margins. Article 6 almost as long as article 2 (ratio: 74:78), at inner margin with one spine, at outer margin with one median plumose seta, nail shorter than pedestal.

Pleopods with 2 retinacula, pilosity of peduncles like that in male.

Uropod 1 peduncle with dorsoexternal row of spines, dorsointernal row absent (except distal spine); inner ramus rather shorter than peduncle, with one lateral spine and pair of simple setae, as well as with 4 distal short spines (Fig. 3D); outer ramus nearly as long as inner ramus, with 4 distal short spines, along margin with one spine and 2 simple setae.

Uropod 2: peduncle with lateral and distal spines; inner ramus slightly longer than outer one, with 2 lateral spines and one simple seta, as well as with 4 distal short spines (Fig. 3E).

Uropod 3 slender but relatively short: peduncle with 2 very small lateral setae, one lateral and 3-4 distal spines. Inner ramus very short, scale-like, much shorter than peduncle, provided with 3 distal simple setae. Outer ramus 2-articulated: first article along outer and inner margin with short spines mixed along inner margin with single short plumose setae; second article rather longer than half of first article, bearing several lateral and distal simple setae (Fig. 3F).

Telson like that in male.

Coxal gills like these in male. Oostegites broad, with marginal setae.

## *NIPHARGUS VLKANOVI BURGASI*, ssp. nov.

Figs. 4-9; 10 A-C

**LOCUS TYPICUS**: Spring near village Varovnik, district Burgas, Bulgaria.

**MATERIAL EXAMINED**:

S-7442 (BU-1)= Fontaine "Golema Vris", ville Malko Tarnovo, district Burgas, 24.4.1972, 6 exp. (leg. S. Andreev);

BU-2= Spring near village Varovnik, district Burgas, 16.10,1975, 10 exp. (leg. S. Andreev), male 15.5 mm, female 12.5 mm (holotype and paratypes);

BU 3= Fountain Petrova niva, village Stoilovo, district Burgas, 24.6.1980, 18. exp, (leg. S. Andreev);

BU-6= Krvatchi, near village Zvezdetz, district Burgas, 3.3.1971, 18 exp. (leg. S. Andreev).

**DIAGNOSIS**: Rather similar to *N. vlkanovi vlkanovi.* Maxilla 1 inner plate with 2-3 setae; coxae rather longer. Gnathopods 1-2 with higher number of lateral transverse row of setae on propodus, telson more spiniferous, inner ramus of uropod 1 in males without bunches of simple setae, single setae mixed with single short spines; epimeral plate 3 more angular to almost acute in both sexes.

**DESCRIPTION: Male 15.5 mm (BU-2):** Body moderately strong, metasomal segments 1-3 with 4 dorsoposterior marginal setae (Fig. 6C); urosomal segment 1 on each dorsolateral side with 1 seta, urosomal segment 2 on each dorsolateral side with 3 setae, urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle.

Epimeral plates 1-2 almost subrounded, with convex posterior margin and marked ventroposterior strong corner- seta; epimeral plate 3 obtusely angular, with marked ventroposterior strong spine and nearly vertical posterior margin in distal part; posterior margin of epimeral plates with several short setae, plates 2 and 3 with 3 ventral spines (Fig. 6C).

Head with short rostrum and subrounded short lateral cephalic lobes, ventroanterior excavation developed (Fig. 4A).

Antenna 1 reaching nearly half of body-length; peduncular articles 1-3 progressively shorter and slender (ratio: 62:45:20), very scarcely setose (Fig. 4B); main flagellum consisting of 18 scarcely setose articles (most of them with one short aesthetasc). Accessory flagellum short, 2-articulated, reaching nearly half of peduncular article 3 (Fig. 4B).

Antenna 2 moderately slender; peduncular article 3 with distoventral bunch of setae reaching length of article itself; article 4 rather longer than article 5 (ratio: 68:58), with 3-4 bunches of ventral setae (the longest setae rather exceeding diameter of article itself), at dorsal margin groups of setae are shorter (Fig. 4C). Article 5 with 4 ventral groups of setae longer than diameter of article itself, dorsal bunches of setae is shorter. Flagellum rather longer than last peduncular article, consisting of 12 articles (Fig. 4C). Antennal gland cone short (Fig. 4C).

Mouthparts well developed. Labrum broader than long, with straight of poorly concave distal margin (Fig. 6A). Labium broader than long, inner lobes well developed, outer lobes entire (Fig. 6B).

Mandible well developed; right mandible: incisor with 4 teeth, lacinia mobilis serrate. Right mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth. Palpus mandible 3-articulated: first article naked, second article with 11 setae; third article nearly as long as second one, with nearly 20 D-setae and 6 E-setae, on outer face with one group of 8 A-setae (Fig. 4D), on inner face with 4 bunches of B-setae (Fig. 4E).

Maxilla 1: inner plate with 3 setae, outer plate with 7 spines (6 spins with one lateral tooth, inner spine with 3 lateral teeth); palpus 2-articulated, not reaching distal tip of outer plate spines, bearing 6-7 distal setae (Fig. 4F).

Maxilla 2 with lobes bearing numerous distal setae, inner plate with several distolateral setae (Fig. 4G).

Maxilliped: inner plate short, with 5 distal spines; outer plate reaching nearly half of palpus article 2, bearing row of distomesial spines (Fig. 7A). Palpus article 3 with 2 medial groups of setae along outer margin, article 4 with bunch of 2 median setae at outer margin, and one seta at inner margin near basis of the nail.

Coxae moderately short; coxa 1 rather broader than long (ratio: 55:39), with subrounded ventroanterior corner bearing 8 marginal setae (Fig. 5A). Coxa 2 nearly as long as broad, with 9 marginal setae (Fig. 5E). Coxa 3 broader than long (ratio: 62:55), with 10 marginal setae (Fig. 6D).

Coxa 4 nearly as long as broad, with very shallow ventroposterior lobe, bearing 9 marginal setae (Fig. 6F). Coxa 5 shorter than coxa 4, broader than long (ratio: 68:40), bilobed, anterior lobe not elongated (Fig. 7B). Coxa 6 broader than long (ratio: 60:30), anterior lobe smaller than that of coxa 5 (Fig. 7C). Coxa 7 shallow, entire, much broader than long (ratio: 56:25) (Fig. 7D).

Gnathopods 1-2 of moderate size, nearly as broad as corresponding coxa. Gnathopod 1: article 2 at anterior margin with row of long single setae, along posterior margin with bunches of long setae; article 3 with one distoposterior bunch of setae (Fig. 5A); article 5 shorter than propodus (ratio: 36:50), at anterior margin with distal bunch of setae). Propodus (article 6) trapezoid, rather longer than broad (Fig. 5B) along posterior margin with 9 transverse rows of setae. Palm slightly convex, inclined nearly to the half of propodus-length, defined on outer face of left propodus by corner S-spine accompanied laterally by 2 slender serrate L-spines and 5-7 corner facial M-setae, on inner face by one subcorner R-spine (Fig. 5C). Palm of right propodus with 2 S-spines, 3 L-spines and one R-spine (Fig. 5D) (probably malformation of right propodus palm). Dactylus reaching posterior margin of propodus, along outer margin with 8 mainly single median setae, at inner (mesial) margin with several short submarginal setae.

Gnathopod 2 is remarkably larger than gnathopod 1; article 2 with row of single long setae along anterior margin and bunches of long setae along posterior margin; article 3 with one distoposterior bunch of setae; article 5 rather shorter than propodus (ratio: 49:62), with distoanterior bunch of setae (Fig. 5E). Propodus trapezoid, nearly as long as broad, along posterior margin with 11 transverse rows of setae (Fig. 5 F); palm straight in proximal part and convex in distal one, on outer face defined by strong corner S-spine accompanied laterally by 2 L-spines and 4 corner facial M-setae (Fig. 5G), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with row of 8 single median setae, along inner margin with several short submarginal setae.

Pereopods 3-4 moderately strong. Pereopod 3: article 2 with row of anterior marginal setae and bunches of setae along posterior margin. Articles 4-7 of different length (ratio: 61:34:41:15), articles 4 and 5 with 3 bunches of setae at posterior margin (setae are shorter to longer than diameter of article itself), along anterior margin with 2-3 groups of setae (Fig. 6D); article 6 at posterior margin with row of short single spines mixed often with short seta; dactylus short and strong, at inner margin with one strong spine near basis of the nail, at outer margin with one median seta nail shorter than pedestal (Fig. 6E).

Pereopod 4 similar to pereopod 3 but rather less setose. Article 2 with row of shorter setae along anterior margin and long setae along posterior margin. Articles 4-7 of different length (ratio: 55:35:43:15), article 5 along posterior margin with 3 groups of short setae and single short spine; article 6 along posterior margin with 5 groups of short setae mixed with single short spine (6F). Dactylus like that of pereopod 3, with one strong spine at inner margin near basis of the nail, nail rather shorter than pedestal.

Pereopods 5-7 rather strong, not elongated. Pereopod 5 distinctly shorter than pereopods 6 and 7, article 2 dilated, rather longer than broad (ratio: 73:50), anterior rather convex margin with 6-7 groups of short setae, along posterior rather convex margin with 14 short setae, ventroposterior lobe not fully developed (Fig. 7B). Articles 4-7 of different length (ratio: 44:50:58:15), articles

4-6 along both margins with groups of short setae mixed often with single short spines. Article 2 is longer than article 6 (ratio: 73:44). Dactylus strong, at inner margin with spine, at outer margin with median plumose seta, nail much shorter than pedestal.

Pereopod 6: article 2 remarkably longer than broad (ratio: 87:51), anterior margin convex proximally, bearing 7 groups of spine-like setae] posterior straight margin with nearly 17 short single setae, ventroposterior lobe not developed (Fig. 7C). Articles 4-7 of different length (ratio: 60:75:91:25), articles 4-6 along both margins with bunches of short spines often mixed with single short setae. Article 6 is longer than article 2 (ratio: 91:87). Dactylus with one spine at inner margin and one median plumose seta at outer margin, nail shorter than pedestal.

Pereopod 7: article 2 longer than broad (ratio: 83:53), along anterior margin with 6 single or groups of spine-like setae, along posterior, rather convex margin, with 14 short setae, ventroposterior lobe not developed (Fig. 7D). Articles 4-7 of different length (ratio: 50:78:98:25), articles 4-6 along anterior and posterior margin with single of paired spines mixed usually with single short setae. Article 2 is rather shorter than article 6 (ratio: 98:83). Dactylus strong, with one strong spine at inner margin near basis of the nail, along outer margin with one median plumose seta, nail shorter than pedestal (Fig. 7E).

Pleopods 1-3 with 2 retinacula. Peduncle of pleopod 1 with one distoanterior seta (Fig. 6G), peduncle of pleopod 2 naked (Fig. 6H), peduncle of pleopod 3 with one posterior seta (Fig. 6 I).

Uropod 1 elongated, peduncle with dorsoexternal rows of spines and dorsointernal row of setae (except distal spine); inner ramus elongated, slightly curved, with 4 spines along dorsal margin and 3 bunches of short simple setae along distoventral margin, at tip with 4 short spines (Fig. 4H); outer ramus reaching nearly half of inner ramus-length, with 2 lateral bunches of simple setae and 4 distal short spines.

Uropod 2: peduncle with several lateral and distal short spines, inner ramus with 4 lateral and 5 distal short spines, outer ramus distinctly shorter than inner one, with one lateral and 4-5 distal short spines (Fig. 4 I).

Uropod 3 long and narrow, peduncle poorly more than two times longer than broad, with single setae and spines; inner ramus short only rather shorter than peduncle (?senior), with 3 distal simple setae and one spine (Fig. 4J). Outer ramus 2-articulated, both article narrowed, of subequal length; first article with several single short spines and setae along inner (mesial) margin; along outer margin with 4 bunches of short simple setae mixed with single short spine; second article along both margins with 1-3 short simple lateral setae and distal bunch of simple setae.

Telson only slightly broader than long, broad, incised nearly 2/3 of telson-length; each lobe tapering distally, bearing 3 short distal spines, 2 single outer marginal spines (exceptionally one spine and one seta), at inner (mesial) margin with 0-1 spine; a pair of short pectinate setae appear near the middle of outer margin of lobes (Fig. 6J).

Coxal gills ovoid, almost reaching distal tip of corresponding article 2 in gnathopod 2 and coxa 4 (Fig. 5E, 6F), shorter in pereopod 3, 5 and 6. (Figs. 6D; 7B, C)

**FEMALE 12.0 mm** with setose oostegites:

Body moderately strong, metasomal segments with 4-5 dorsoposterior marginal setae; urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 with 3 spines on each dorsolateral side, urosomal segment 3 naked.

Epimeral plates rather more pointed regarding males (Fig. 8E), epimeral plate 2 with 2 ventral spines, epimeral plate 3 with 3 ventral spines.

Antennae 1 and 2 like these in male, main flagellum consisting of nearly 24 articles; flagellum of antenna 2 consisting of 11 articles, antennal gland cone short.

Mouthparts like that in male. Mandibular palpus article 1 naked, article 3 with nearly 25 D-setae and 6 distal E-setae, on inner face with 8 B-setae sitting in 3 groups (3-3-2) (Fig. 9A), on outer face one group of 5 A-setae (Fig. 9B). Maxilla 1 inner plate with 3 setae. Maxilliped inner plate with 5-6 distal spines, palpus article 4 at inner (mesial) margin with 2 setae near basis of the nail.

Coxae 1-4 rather longer than these in male Coxa 1 rather broader than long (ratio: 53:46), with subrounded ventroanterior corner and bearing 10 marginal short setae (Fig. 8A). Coxa 2 longer than broad (ratio: 63:56), bearing 10 short marginal setae (Fig. 8B); coxa 3 longer than broad (ratio: 70:58), with 8-9 marginal setae (Fig. 8C); coxa 4 only slightly longer than broad (ratio: 67:60), with 7-9 marginal setae, ventroposterior lobe is not distinctly developed (Fig. 8D). Coxa 5 broader than long (ratio: 52:39) (Fig. C); coxa 6 broader than long (ratio: 48:34) (Fig. 9D); coxa 7 shallow, like that in male (Fig. 9E).

Gnathopods 1-2 similar to these in male but slightly smaller. Gnathopod 1 article 5 shorter than propodus (ratio: 70:63), with distoanterior bunch of setae. Propodus trapezoid, rather longer than broad (ratio: 70:63), along posterior margin with 7 transverse rows of setae (Fig. 8F); palm slightly convex, inclined nearly to the half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 3 slender L-spines and 3 facial corner M-setae, on inner face by one subcorner R-spine. Dactylus along outer margin with row of 7 single median setae, along inner margin with several short setae.

Gnathopod 2 moderately larger than gnathopod 1; article 5 shorter than propodus (ratio: 70:82), with distoanterior bunch of setae. Propodus trapezoid, nearly as long as broad, along posterior margin with 9 transverse rows of setae (Fig. 8G); palm slightly convex, inclined rather over half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 L-spines and 3-4 corner facial M-setae, on inner face by one S-spine. Dactylus along outer margin with 7 single or paired median setae, along inner margin with several short setae.

Pereopods 3-4 like these in male. Pereopod 4: articles 4-7 of different length (ratio: 45:37:43:14), articles 3-6 scarcely setose. Dactylus with one spine at inner margin and one median plumose seta at outer margin (Fig. 8D).

Pereopod 5 shorter than pereopods 6-7, article 2 rather longer than broad (ratio: 65:45), anterior and posterior margin moderately convex, anterior margin with 7 single or paired spine-like setae, posterior margin with 12 setae (Fig. 9C), ventroposterior lobe not fully developed. Articles 4-7 of different length (ratio: 34:40:46:16). Articles along both margins with single or paired setae accompanied often with single or groups of short spines. Article 2 longer than article 6 (ratio: 65:46), dactylus with strong spine at inner margin and one median plumose seta at outer margin, nail shorter than pedestal.

Pereopod 6: article 2 rather longer than broad (ratio: 73:51), both margins slightly convex; along anterior margin with 7 single spine-like setae and distal group of setae, along posterior margin with 18 short setae, ventroposterior lobe marked (Fig. 9D). Articles 4-7 of different length (ratio: 45:61:73:18), article 4 with 3 single or groups of posterior spines, along anterior margin with 3-4 single or groups of setae; articles 5-6 along both margins with several groups of short spines and setae. Article 2 nearly as long as article 6. Dactylus short, at inner margin with spine near basis of the nail, at outer margin with one median plumose seta, nail shorter than pedestal.

Pereopod 7: article 2 longer than broad (ratio: 75:52), with rather convex lateral margins, at anterior margin appear 6-7 single spine-like setae and distal bunch of setae, along posterior margin with 13-14 short setae (Fig.9E) ventroposterior lobe marked. Articles 4-7 of different length (ratio: 42:59:80:22), articles 4-6 along both margins with single or groups of spines often mixed with short setae (spines and setae not extend diameter of article itself); article 2 shorter than article 6 (ratio: 75:80). Dactylus at inner margin with spine, at outer margin with one median plumose seta (Fig. 9F), nail shorter than pedestal.

Uropod 1 peduncle longer than rami, with dorsoexternal row of spines, dorsointernal row is composed of one spine, one spine-like seta and one seta (in proximal part); rami of nearly equal length, inner ramus with 2 dorsal median and 5 distal short spines, at ventral margin with one bunch of simple setae mixed with one spine and one short median seta; outer ramus at dorsal margin with 3 median and 5 distal short spines, at ventral margin with 2 groups of simple setae mixed with one spine each (Fig. 9G).

Uropod 2: rami nearly equally long, with single lateral and 5 distal short spines, sometimes mixed with one short simple seta (Fig. 9H).

Uropod 3 shorter than that in male. Peduncle almost twice as long as broad, inner ramus scale-like, short, with distal spine. Outer ramus 2-articulated: first article elongated, along outer margin with bunches of short spines, along inner (mesial) margin with single or paired spines mixed with one longer plumose seta (Fig. 9 I); second article much shorter than first one (ratio: 25:107), along both and tip with simple setae.

Telson almost as long as broad (ratio: 74:75), incised nearly ¾ of telson-length; each lobe with 3 distal spines (the longest spine reaching rather less than half of telson-length), and one median spine at outer margin, inner marginal and facial spines absent; a pair of short plumose setae is attaches near the middle of outer margin (Fig. 8H).

Coxal gills ovoid, of moderate size, not reaching ventral margin of corresponding article 2 (Fig. 9C). Oostegites broad, with long marginal setae.

**DERIVATIO NOMINIS:** The subspecies *N. vlkanovi burgasi* is nominated based on locality district name, Burgas.

**HOLOTYPE:** male 15.5 mm; **paratype:** female 12.0 mm. Holotype is deposited in Karaman`s Collection in Podgorica, Montenegro.

**VARIABILITY**:

The specimens from Burgas region differs remarkably from *N. vlkanovi* of type-locality (see key). Despite that we have at disposition for study holotype and paratype of *N. vlkanovi (*from Sofia region) only, we found row of morphological different characters of specimens from Burgas region regarding these of ssp. *vlkanovi* from Sofia region.

Within the ssp. *burgasi*, there are rather variability of telson: the number of strong spines along outer margin (1-3 spines) (Fig. 10A, C), along inner (mesial) margin (0-1) (Fig. 8H, 10A, C), shape of epimeral plate 3 from poorly angular to distinctly acute (Figs. 6J, 10B, D) and number of ventral epimeral spines (2-4), size of adult males and females (11-20 mm), more or rather less inclined palm of gnathopod`s propodus, number of outer marginal median setae on gnathopod`s dactylus.

When we have in hands scarce material of ssp. *vlkanovi* with unknown variability, we couldn`t consider the specimens from Burgas region as identic with these from Sofia region. By this way, we established the specimens from Burgas region (Black Sea area) as new subspecies, ***Niphargus vlkanovi burgasi*, ssp. nov**. with type locality: Spring near village Varovnik, Burgas, Bulgaria.

But we can`t exclude the possibility that morphological characters of specimens from Burgas region are within the maximal limits of variability of *N. vlkanovi*, and discovery of new samples from different localities will put more light on this problem.

On the other hand, *N. vlkanovi* is rather similar to the species *Niphargus anatolicus* S. Karaman 1950b, known from locus typicus only [Emirgan (N. off Istanbul, European part of Turkey). We recently partially redescribed and figured this species (G. Karaman, 2024a), but it is necessary to collect new material of *N. anatolicus* and *N. vlkanovi* from type-locality to clear their taxonomical position.

## *NIPHARGUS (Niphargopsis) TRISPINOSUS* Dancau & Capuse, 1959

*Niphargopsis trispinosus* Dancau & Capuse, 1959: 1, figs. 1-4; Andreev, 2001: 86;

*Niphargopsis trispinosus* (part) G. Karaman, 1982: 89, figs. I-VII.

**LOCUS TYPICUS**: Mehadia, Timisoara region, Romania).

**LOCALITIES CITED**:

Andreev, 2001: Bulgaria: well in village Glavatzi, reg. Montana.

**DISTRIBUTION**

The specimens determined sub name *Niphargopsis caspary* (Pratz 1866) [locus typicus: München, Germany] are mentioned by various authors from France, Germany, Switzerland, Austria, Serbia, along the coast of the Sea in Middle Tertiary, from Rhona River in France, over Switzerland and Germany along present valey of Danube River.

The similar specimens, but determined sub name *Niphargopsis trispinosus* by Dancau & Capuse, 1959, are described from Romania and Bulgaria. Some authors cited *Niphargopsis trispinosus* Dancau & Capuse, 1959 as member of genus *Niphargus* Schiödte, 1849.

**DISCUSSION**

G. Karaman (1982) redescribed *N. caspary* based on specimens from France, Serbia and Romania, showing absence of significant morphological differences between various localities of these countries, considering specimens from Romania (*N. trispinosus*) identic with these of other countries (*N. caspary*). The new molecular genetic methods will probably help in to resolve taxonomical status and relations between these two species.

The presence of the same subterranean species on very long distances is already known in various subterranean species: *Niphargus valachicus* known from Germany to Turkey and Iran (G. Karaman, 1998c); *Niphargus gallicus* Schellenberg, 1935, known from S. France and Romania, etc.

Despite the absence of known significant morphological differences between various distant populations of one species, for the moment we don`t know its detailed molecular, genetic and other characters and possible clear differences which conduct to the possible reproductive isolation of these distant populations. The best way to resolve this problem is to study the degree of reproductive isolation in crossing experimental conditions specimens of various populations.

***NIPHARGUS* (*Phaenogammarus*) *VALACHICUS* Dobreanu & Manolache, 1933**

[Synonymy is shortened]

*Niphargus tatrensis valachicus* Dobreanu & Manolache, 1933: 104, figs. 2-4;

*Niphargus valachicus* S. Karaman, 1934: 332; G. Karaman, 1973: 150, fig. 4; Sket, B., 1981: 88;

*Niphargus (Phaenogammarus) valachicus* S. Karaman, 1960: 83;

*Niphargus (Supraniphargus) valachicus valachicus* S. Karaman, 1950c: 68, figs. 35-37; Sket, 1958: 67;
*Niphargus (Phaenogammarus) mediodanubialis* Dudich, 1941: 61, figs. 1-2;
*Niphargus (Phaenogammarus) mediodanubialis f. aschizotelzon* Dudich, 1941: 72, fig. 3;
*Niphargus mediodanubialis = N. valachicus* S. Karaman, 1950a: 14;
*Niphargus ivanovi* nom. nudum, Schäferna in lit., G. Karaman, 1974: 27; Barnard, J.L. & Barnard, C.M., 1983: 692.

**LOCUS TYPICUS**: Bucharest, Romania.
**LOCALITIS CITED**: BULGARIA:
G. Karaman 1973: Devnja, mixed with *Gammarus* sp.
**DISTRIBUTION**:
Along the Danube River valley, from Germany, Austria, Hungary, Slovenia, Croatia, Bosnia & Herzegovina, Serbia, Romania, SE Ukraine, Turkey, etc. to Iran.
**REMARKS**.
*Niphargus valachicus* has unique form of urosomal segments [among several hundred taxa of genus *Niphargus*], with extremely large distribution regarding other *Niphargus* taxa. For this reason I often doubt to recognize the subgeneric status of this species [subgenus *Phaenogammarus* Dudich, 1941] or not, because deep molecular genetic investigations on specimens from numerous remote localities are not provided; on the other hands, new more advanced similar studies in the future will put more light on it, as well as on the general position of subgenera and subspecies categories, recognized in our Zoological Codex.

## KEY TO THE *NIPHARGUS* SPECIES OF BULGARIA

1. Lobes of telson with 3 long distal plumose setae; propodus of gnathopods 1-2 remarkably egg shaped ..........................................................................2
-- Lobes of telson without long distal plumose setae; propodus of gnathopods 1-2 more or less trapezoid or *kochianus*-type
2. Dactylus of gnathopods 1-2 at outer margin with one median seta; maxilla 1 inner plate with one seta; telson narrow...........................................*JOVANOVICI*
--- Dactylus of gnathopods 1-2 at outer margin with 3 or more median setae; maxilla 1 inner plate with 2 setae; telson gaping ........................... *DOBROGICUS*
3. Maxilla 1 outer plate dilated, bearing numerous (over 30) pectinate spines *Niphargus* (*Niphargopsis*) *TRISPINOSUS* (=? *CASPARY*)
--- Maxilla 1 outer plate not dilated, bearing 7 distal spines..................................4
4. Dactylus of gnathopods 1-2 with one median seta along outer margin ..............5
--- Dactylus of gnathopods 1-2 with row of median setae along outer margin.....11
5. Pleopods 1-3 with 2 retinacula...................................................*MELTICENSIS*
--- Pleopods 1-3 with 3 or more retinacula ..........................................................6
6. Epimeral plates 1-3 pointed .........................................................................7
--- Epimeral plates more or less subrounded .......................................................8

7. Article 2 of pereopods 5-7 broadly convex, with well-developed ventroposterior lobe; propodus of gnathopods 1-2 kochianus type, with palm not inclined........................................................................................................ *DECUI*
--- Article 2 of pereopods 5-7 more or less elongated, without ventroposterior lobe; propodus of gnathopods 1-2 trapezoid, with remarkably inclined palm.......... ............................................................................................................ *GEORGIEVI*
8. Maxilla 1 inner plate with 1 seta.......................................................................9
--- Mx1 inner plate with 2 setae ........................................................... *CVETKOVI*
9. Peduncle of pleopods 1-3 with numerous lateral setae ........................................ ..........................................................................*PECARENSIS PECARENSIS*
--- Peduncle of pleopods 1-3 scarcely setose, bearing 0-3 single setae each........10
10. Telson of male in with numerous long distal spine-like setae; females with uropod 1 rami not inflated.......................................... .*KRAGUJEVENSIS REMUS*
--- Telson in male with short spines and spine-like setae; female with inflated uropod 1 rami.......................................................*KRAGUJEVENSIS FEMINEUS*
11. Dactylus of pereopods with elevated number of spines along inner margin...12
--- Dactylus of pereopods with only one spine or spine-like seta along inner margin……………………………………………………………………….......13
12. Urosomal segments 1-2 with one very strong spine on each dorsolateral side... ......................................................*Niphargus* (*Phaenogammarus*) *VALACHICUS*
--- Urosomal segments 1-2 with one or more setae or weak spines. *BULGARICUS*
13. Pleopods with elevated number of retinacula (up to 5)........... *CEPELARENSIS*
--- Pleopods with 2, exceptionally 3 retinacula only............................................14
14. Uropod 3 in males and females short, with short distal article of outer ramus. Article 2 of pereopods 5-7 with lateral margins broadly convex, with short ventroposterior lobe [mx1 inner plate with 4 setae, epimeral plates acute].............. ........................................................................................................ *MERIDIONALIS*
--- Uropod 3 in males long, with long distal article of outer ramus; article 2 of pereopods 5-7 with straight or less convex lateral margin...................................15
15. Uropod 1 inner ramus in both sexes as long as outer ramus (telson without facial spines) ............................................................................................ *BURESCHI*
--- Uropod 1 inner ramus in male much longer than outer ramus (telson with or without facial spines) .........................................................................................16
16. Telson without lateral spines, facial spines present. Epimeral plate 3 slightly pointed,; article 2 of pereopod 7 shorter and stouter, broad; maxilla 1 palpus shorter, reaching basis of outer plate spines ................................... *TOPLICENSIS*
--- Telson with lateral spines, facial spines absent. Epimeral plate 3 slightly angular or obtuse, article 2 of pereopod 7 longer and more narrow; maxilla 1 palpus slightly exceeding basis of outer plate spines..........................................17
17. Inner ramus of uropod 1 in males with numerous lateral simple setae. Maxilla 1 inner plate in male with 1 seta, in female with 1-2 setae; coxae 1-4 in males shallow. Propodus of gnathopods 1-2 in both sexes with lower number of transverse rows of setae (5-6). Uropod 1 peduncle without dorsointernal row of setae (except distal spine) in both sexes. Epimeral plate 3 in male obtusely angular or subrounded...................................................... *VLKANOVI VLKANOVI*
--- Inner ramus of uropod 1 in males with single short setae mixed with spines. Maxilla 1 inner plate in both sexes with 2-3 setae; coxae 1-4 in males rather

longer. Propodus of gnathopods 1-2 in both sexes with elevated number of transverse rows of setae (8-11). Propodus of gnathopod 1 in females remarkably smaller than that of gnathopod 2; Uropod 1 peduncle with dorsointernal row of setae. Epimeral plate 3 in male distinctly angular or slightly pointed .................... ............................................................................................ *VLKANOVI BURGASI*

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**- Abstract**

The summary, in English language, should provide basic data on the problem that was treated and the results obtained. It should be brief, preferably one paragraph only, up to 250 words, but sufficient to inform the reader of the character of the work, its results and its conclusions. Include the keywords and phrases you repeated in your abstract.

**- Key words**

Keywords should provide 4-6 words or compound words, suitable for an information retrieval system. Choose the appropriate keywords and phrases for your article. Think of a phrase of 2-4 words that a researcher might search on to find your article. Repeat your keywords and phrases 3-4 times throughout the abstract in a natural, contextual way.

**Main text of the manuscript includes the following sections:**

**- INTRODUCTION**

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

**- MATERIAL AND METHODS**

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.

**- RESULTS** followed by **DISCUSSION**

Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interprets results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

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)**

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. More details you can find in the Annex to the INSTRUCTIONS TO AUTHORS / Bibliographic style on the web page of the Journal: www.agricultforest.ac.me.

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