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Anissa GARA[1,2*], Dorra AJABI [1]

EFFECT OF ANIMAL HUSBANDRY ON ENVIRONMENTAL PROFICIENCY

SUMMARY

Sustainable agriculture is an application of the concept of sustainable development to the agricultural sector and more specifically at the farm level. It takes into account the three dimensions - economic, social and environmental - in a global framework. It is in this context that this study aims to assess the effect of the integration of livestock into the production system on the sustainability of farms in the Mornag region, located in the northeast of Tunisia. Two groups of farms were derived: with livestock and without livestock. The Farm Sustainability Indicators (IDEA) method was used to meet this research objective. From the analyses of the previous results and the comparisons between the means of the indicators of each component according to the mode of production, we were able to deduce that this factor considerably influences agricultural sustainability on both agro-ecological and socio-territorial scales. Indeed, the group of farms that include livestock have better averages in most indicators. These results could be explained by the fact that the association of crops with livestock within a production unit is considered an asset in the sustainability of an operation since it allows positive interactions and synergies between the different elements of the system. Indeed, in the mixed crop-livestock system, the diversification of production, crop rotation and the use of animal manures contribute to improving soil fertility. In addition, animal husbandry makes it possible to diversify income and distributes it over time and, therefore, ensures a certain stability in the economy. Some farmers even have a daily income from animal products such as eggs and chickens; others take advantage of some products for family consumption.

Keywords: Sustainability, Farms, Livestock, Tunisia

INTRODUCTION

Applied to the field of agriculture, the concept of sustainable development implies that the operator's concerns no longer relate solely to the objective of achieving economic profitability, but takes into account other dimensions, namely

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respect for the environment (preservation and good management of non-renewable resources, of the biodiversity of ecosystems and landscapes, optimization of production factors, etc.), social integration (promoting the integration of the farmer, integrating an ethical dimension, ensuring a certain social equity and well-being for the farmer, etc.), the viability and transferability of the farm (the economic efficiency of the operating system, financial autonomy and dependence on aid, transferability of capital and transmission of knowledge, etc.)(Massin et al. 2016). Agriculture that simultaneously takes into account the three dimensions makes it possible to generate sufficient income for the household, it applies agricultural practices that do not affect the environment, it contributes to job creation and the social integration of farmers, and it is transmissible, so it can be qualified as "sustainable agriculture". Landais (1998) indicates that sustainable agriculture is economically and socially viable agriculture, ecologically reproducible and transmissible. However, this growing need to integrate and assess the new concept of sustainability within farms has prompted the scientific community to question the methods and tools that make it possible to assess a production system and translate sustainability of the three dimensions in an operational way. Indeed, for an assessment to be relevant it must be based on reliable indicators that describe the main aspects of an operating system, namely environmental, economic and social. In this context, several assessment methods have been developed since the 1990s and they are based on a set of indicators covering certain aspects that assess sustainability within a farm. In Tunisia, the evaluation of sustainability is a relatively recent subject and the tests carried out in this field, despite their importance and usefulness, remain limited and they have not gone beyond the descriptive character and the diagnostic aspect (Laajimi et al., 2009). Similarly, Nabradi (2011) affirms that: "Utilizing livestock in agriculture often improves the sustainability of the system of an environmental (ecological), economic, and social viewpoint. Animal production can be economically sustainable because of its role in trade, market and feed supply disruptions, as it diversifies the activities of producers, decreases controls risk at the farm and national levels, enhances farm maintenance and increases the possibilities for employing the rural population".

Likewise, this study constitutes an assessment of the level of sustainability of different farms in Tunisia, in the region of Mornag, delegation of Ben Arous. The study region is a periurban region located at 10 km from the down-town of the capital Tunis. therefore, we remark a conflict between rural and grilcultural activities comprsing animal husbandy and urbalization. The latter is conquering fertile land at a rapid rhythm. In accordance with Parsipour et al. (2019): "We note Peri-urban environments are some spaces where are strongly under effect of daily growth of urban population and experience constitutional changes. Firstly, their land use which turns from agricultural and husbandry into residential use and secondly they merge with urban fabric".

The goal of this research is to find out the effect of livestock integration on farm sustainability. Hence, two groups of farms are derived: farms with livestock and farms without livestock (only crop farming system). It is with this in mind that the methodology adopted for this study has three stages: the farmers' inquiry and data collection then IDEA tool application and results discussions.

MATERIAL AND METHODS

Data collection

The Mornag delegation is located in the governorate of Ben Arous in northern Tunisia and covers an area of 6,900 ha. Enjoying a Mediterranean climate, the average temperature varies between 11°C in winter and 26°C in summer. The Mornag delegation is predominantly fruit-arboriculture (65%). It is essentially a communal region and the rate of urbanization exceeds 90% of the total population of the governorate in 2009 (Ministry of Environment, 2011). Moreover, this pole continues to record urban growth in the form of housing estates sometimes to the detriment of natural and agricultural areas.

Besides, we encountered reluctance on the part of the farmers to answer the questionnaire. This difficulty has worsened, particularly during the confinement period due to the COVID-19 pandemic. Mornag's delegation contains 14 regions (called in Arabic 'Imedat'). The present study concerned only one region, which bears the same name as the delegation: Mornag Imedat or Mornag Region. In this region of Mornag, a number of 29 completed questionnaires were recorded. The sample is exhaustive because it contains farms with breeding and others without breeding in equal shares. The sample represents 5% of the total number of existing farms in the Mornag region.

IDEA scoring

The IDEA method (Indicateurs de durabilité des exploitations agricoles or Farm sustainability indicators) was designed by a multidisciplinary group made up of agronomists, socio-economists and ecologists belonging to various institutions (teaching, research, development). According to Vilain (2003) this method was designed to allow a diagnosis of farm sustainability from direct surveys of farmers. This method is the most suitable for achieving the objective of this work, which is to assess the sustainability of farms. Indeed, this project will allow us to qualitatively assess the sustainability of private farms and development in the study area in order to highlight systems and strategies in favour or not of agricultural sustainability. The indicators of the IDEA method have all been designed in such a way that they can respond, directly or indirectly, to a number of objectives for sustainable agriculture. These objectives are formulated around three scales of sustainability, namely: good management and protection of natural resources (ecological scale), respect for certain qualities of citizenship and a certain social level (social scale), and the guarantee of a good economic and productive function of farm (economical scale). The IDEA method has three scales, which represent the dimensions of sustainability in a production

system. They are of the same weight, the values of which vary between 0 and 100 points, and each scale is subdivided into components which themselves have specific indicators and which characterize a sustainable system. The components bring together 42 indicators. Each characterizes a practice in a sustainability scale and having an assigned and quantified score. The indicators seek to reflect the systemic dimension of the farm, but also each indicator aims to deliver a message in order to identify possible paths of progress towards increased sustainability (Briquel et al, 2010). It is proposed by relying on the indicators of the IDEA method (Indicators of the sustainability of agricultural holdings), which offers operational content to the concept of sustainability at the farm level, to compare the level of farm sustainability based on the factor of farming mode, that is to say animal breeding integrated or not to crop farming. The overall sustainability rating of a farm is the scale with the lowest score. This principle allows for the simultaneity of the three dimensions and therefore an integrated approach to sustainability. The scores obtained for each component will make it possible to identify the factors affecting the sustainability of the operation in the dimension concerned. The higher the score on a scale, the more sustainable the operation is considered on that scale. The minimum score associated with most indicators is zero score. This note can simply mean that the farm is not affected by the indicator. For example, the animal diversity or endangered breeds indicators do not apply to farms that do not have livestock. For the farms concerned, the zero score does not necessarily mean irreversible handicaps to sustainability, but rather that the farm has room for improvement. The maximum scores for each indicator have been set to cap the total number of sustainability units.

RESULTS AND DISCUSSION

In order to better explain these differences, we will study and compare the results of the components within each scale of the three groups of farms according to land size.

Agro-ecological scale

In the agro-ecological scale, we can see that farms that integrate livestock into the production system are more sustainable in all components of the scale (Figure 2). Farms that integrate livestock have very good averages in the Agricultural Practices component, followed by the Diversity component. Farms with a purely plant production system, on the other hand, have very low averages in the two components “Space organization” and “Diversity”.

The “Diversity” component reflects the weight given to the autonomy and diversity of production systems to achieve a model of sustainable agriculture (Table 1). The low averages of farms in the plant production system can be explained by the fact that they are sanctioned with a score of zero in indicator A3: 'Animal diversity', and a low score in indicator: 'Diversity of annual crops' as they generally do not have meadows or fodder crops. More the production system is diversified more sustainable is the agroecology aspect. This is in accordance with

results of assessment of agrocecological sustainability at Souk Ahras in Algéria where Latreche *et al.* (2019) found out that: "The highest sustainability was assigned to cereal other crops, followed by cereal potatoes system; due to good economic performance (high incomes).

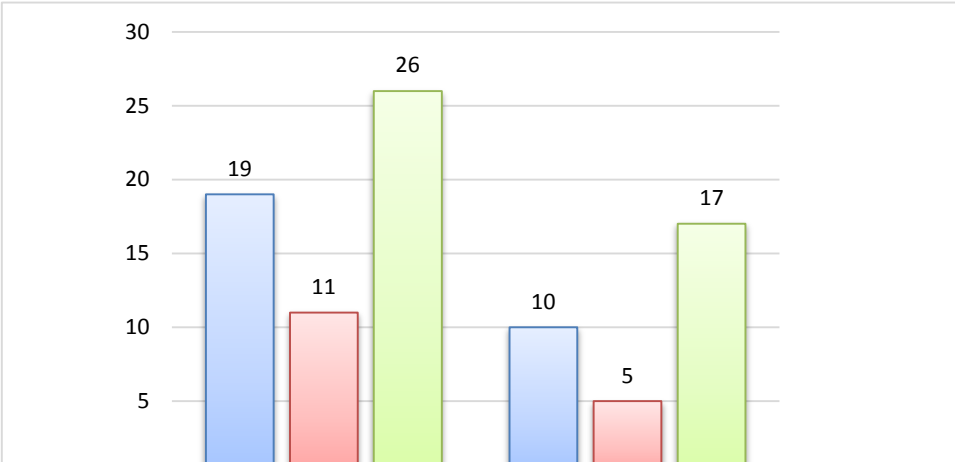

Figure 2. Averages scoring of the components of the agro-ecological scale according to the farming mode.

However, both systems have low agro-ecological sustainability due to increased pesticide use and high energy consumption and treatment frequency; high values of these indicators affect negatively durability. The cereal grain legumes system has a good durability increased by the agro ecological effect linked to the introduction of a leguminous in the rotation.

Table 1. Means of the indicators of the "Diversity" component according to the farming mode.

	Diversity of annual crops (/14pts)	Diversity of perennial crops (/14pts)	Animal diversity (/14pts)	Valorization and conservation of the genetic heritage (/6pts)	Total (/33 pts)
Livestock	4.75	5.25	7	1.86	18.9
Without livestock	3.1	6.9	0	0.1	10.1

This system is very appreciated by the farmers, in addition it concords with the strategy of the State which aims is to replace fallow land with a grain legume. Cereal pastured and worked fallow have low durability with a priori for cereal pastured fallow; but the edapho-climatic conditions, the socio-economic context and the vocation of the region ensure the persistence of these two systems".

This imbalance considerably affects the autonomy and sustainability of the farm. Indeed, the integration of animal breeding offers synergies and complementarities within production systems that make it possible to better develop resources.
Also, the absence of livestock induces low or zero scores in the indicators of the component "Organization of space" having a close relationship with the practice of breeding: 'Management of organic matter' because livestock ensures self-sufficiency in organic matter and maintains soil fertility; 'Contribution to environmental issues' because the protection of certain plant and animal species through compliance with territorial specifications is necessary for the conservation of natural biodiversity and genetic heritage; 'Valuation of space', which is an indicator related to breeding and which assesses the stocking of livestock per area intended for animals and which will, therefore, penalize farms without livestock with a zero score; and forage areas (A11: Management of forage areas), which sanctions farms with a plant production system only because they do not have an area intended for permanent meadows of grasslands or pastures (Hoernlein, 2014) (Table 2).

Table 2. Means of the indicators of the "Organization of space" according to farming mode component.

	Rotation (/8pts)	Parcells dimension (/6pts)	Management of organic matter (/5pts)	Ecological regulation zones (/12pts)	Contribution to environmental issues (/4pts)	Valuation of space (/5pts)	Management of forage areas (/3pts)	Total (/33 pts)
Livestock	1.75	2.625	2.875	0.25	0.625	1.25	1.875	11.25
Without livestock	0.5	2.6	0.9	0.9	0.0	0.0	0.0	5.0

With regard to the 'Agricultural practices' component, farms practicing only vegetable production recorded low scores, in particular because of indicators related to 'fertilization treatments', 'liquid 'organic effluents' and 'pesticides' (Table 3).

Table 3. Averages of the indicators of the 'Agricultural practices' component according to farming mode.

	Fertilization (/8pts)	Liquid organic effluents (/3pts)	Pesticide (/13pts)	Veterinary treatment (/3pts)	Protection of soil resources (/5pts)	Management of water resources (/4pts)	Energy dependency (/10pts)	Total (/34pts)
Livestock	3.75	2.25	6.75	1.125	1.875	3.875	6.25	25.875
Without livestock	0.8	1.9	2.6	0.0	1.8	4.1	6.0	17.1

In fact, monocultures and poorly diversified production systems require treatment. Indeed, the presence of livestock and rangelands delays the harmful effects on the environment and offers several agronomic advantages to the farm.

On the one hand, permanent meadows and areas intended for animals, especially if they are cultivated in a mowing-pasture rotation, improve soil

fertility and slow down runoff. On the other hand, some production systems are more sensitive and, therefore, require excessive use of treatments and pesticides such as viticulture and monocultures, unlike large crops and meadows, which are not as demanding.

Socio-territorial scale

In this scale, we will determine the influence of livestock on the degree of integration of the farm in its territory and in society as well as on the quality of life of the farmer.

The results of the calculations of the means of the three components of the socio-territorial scale for the two groups of farms are presented in figure 3. We can see that the integration of breeding practice has a positive impact on the "Ethics and human development" component and a little less on the "Employment and service" component. On the other hand, this impact is negative in the "Quality of products and terroir" component.

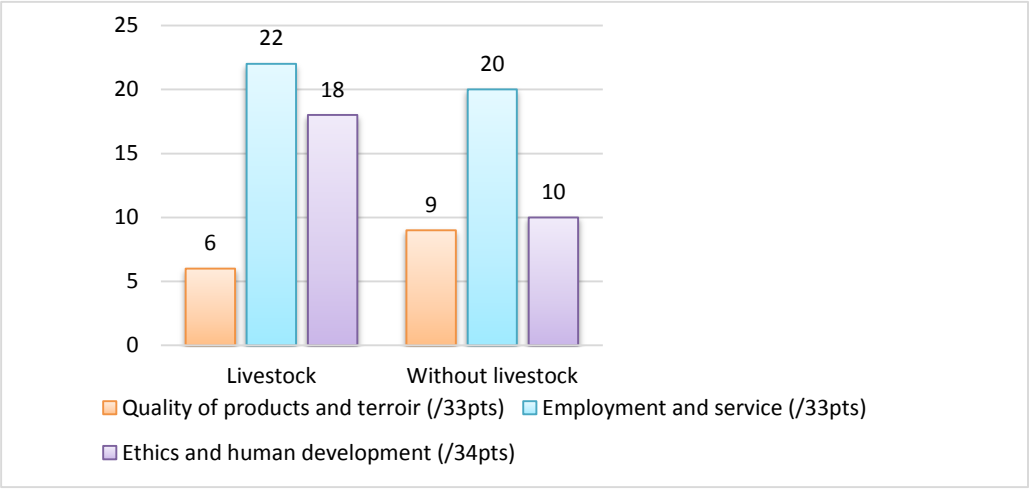

Figure 3. Averages scoring of the components of the socio-territorial scale according to the farming mode.

Regarding the first component 'Ethics and human development' (Table 4), the significant difference between the averages is due to the indicators: 'Contribution to the global food balance' and 'Animal welfare', which sanction the systems of production without breeding. Indeed, the notion of responsibility for the global food balance, illustrated by indicator 'Contribution to the global food balance', considers that the massive use of imported products reinforces the dependence of the farm on the world market to the detriment of the autonomy of resources. The indicator calculates imported area equivalents, which it compares to the farm's useful agricultural area (UAA), (with 4 tons of concentrated

purchased livestock feed are equivalent to 1 ha of UAA). Farms that do not practice animal husbandry, which do not produce protein crops or for which the "import rate" exceeds 50%, will score zero in this indicator. The scores in the other indicators are slightly closer and reflect aspects related to the living, working and training conditions of farmers and employees. This shows that the practice of breeding has no significant effect on the latter.

Table 4. Means of the indicators of the “Ethics and human development” component according to the farming mode.

	Contribution to the global food balance (/10pts)	Animal welfare (/3pts)	Training (/6pts)	Labor intensity (/7pts)	Quality of life (/6pts)	Isolation (/3pts)	Reception, hygiene and security (/4pts)	Total (/34pts)
Livestock	8	2.75	0.25	0.875	3.25	1	2.25	18.375
Without livestock	0.5	0.0	1.6	0.4	3.8	1.5	2.4	10.1

As for the “Employment and services” component, the averages for the two groups come closer with a slightly higher score for holdings engaged in livestock farming (Table 5). Indeed, the first group slightly exceeds the second mainly in the indicator ‘Autonomy and development of local resources’, which assesses the capacity of the operation to be self-sufficient in terms of resources and raw materials. The score for this indicator incorporates the percentage of autonomy in livestock feed, fertilizer, development of local and renewable resources as well as seed autonomy. The score can, therefore, be penalized if the farm does not breed livestock or if it does not have autonomy of at least 50% of cattle feed from the territory. This explains the low score (1.2 / 10) of the second group.

Table 5. Average indicators of the “Employment and services” component according to the farming mode.

	Valuation by short chains (/7pts)	Autonomy and development of local resources (/10pts)	Services and pluriactivities (/5pts)	Contribution to employment (/6pts)	Collective work (/5pts)	Probable continuity (/3pts)	Total (/33pts)
Livestock	6.875	4.875	0.625	5.5	1.125	2.75	21.75
Without livestock	6.7	1.2	1.7	5.5	1.9	2.7	19.7

Finally, the "Product and Terroir Quality" component is the only one to record better averages among the group of farmers who do not practice livestock (Table 6). It is a component that assesses the notion of authenticity of the identity of the terroir (Girardin *et al.*, 1999), the development of buildings and the surroundings of the farm (enhancement of the built heritage and the landscape), as well as the recognition of the quality of products at the national or international

level through labels or certifications (quality approach). The latter indicator has a very low average for the two groups of farms who do not value their products enough due to the lack of required financial resources. The same for indicator B3 "Management of non-organic waste", which has an almost zero value in the two groups, which reflects a general lack of awareness among operators in the Mornag area regardless of the production system.

The concepts of selective sorting, disposal by collection and recovery of waste are almost absent. In addition, farmers seem to practice burning, a practice unfavourable to the environment that the IDEA method sanctions with a negative score (-3pts). This component then reflects an aspect of farmers' practices and commitment that does not depend on the farming system.

Table 6. Average indicators for the 'Product and terroir quality' component according to the farming mode.

	Quality approach (/10pts)	Enhancement of the built heritage and the landscape (/8pts)	Management of non-organic waste (/5pts)	Accessibility of space (/5pts)	Social involvement (/6pts)	Total (/33 pts)
Livestock	0	2.25	0	1.5	2.25	6
Without livestock	0.8	3.1	0.3	2.2	2.2	8.7

Economic scale

The last sustainability scale looks at farmers' practices from an economic perspective. Under market conditions, the farm must generate sufficient current income to ensure the farmer certain autonomy in his choices and to allow him to move towards a sustainability approach (Briquel et al, 2010). From the radar graphic representation of the results (Figure 4), we can see that farms with integrated agriculture have slightly higher averages in the three components: 'Viability', 'Independence', and ‘Efficiency’.

For the "Viability" component, the averages of the two groups are very close and do not really depend on whether or not the practice of breeding is integrated. However, the first group has an acceptable average and a little higher than the second in the indicator 'Economic viability', which reflects a larger gross surplus (Table 7).

For farm independence, the averages are also similar in the two indicators ‘Financial autonomy’ and ‘Aid sensitivity’ with a slight advantage for the first group. For the third component ‘Transmissibility’, the two groups recorded low averages due to too much capital or the absence of partners and family labour; which represents a handicap to resuming operations in the event of cessation of activity or the departure of the manager.

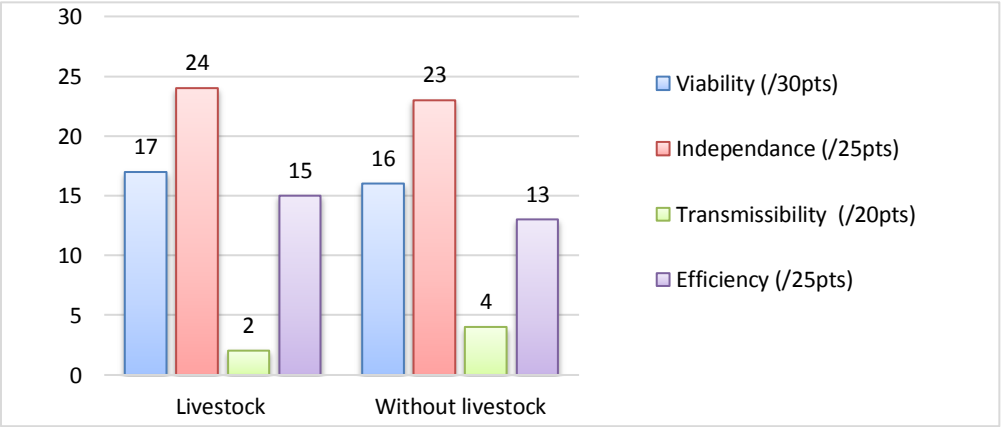

Figure 4. Averages scoring of the components of the economic scale according to the farming mode.

Table 7. Scoring of economic scale indicators according to farming mode

	Viability			Independence			Transmissibility		Efficiency	
	Economic viability (/20pts)	Economic specialization rate (/10pts)		Financial autonomy (/15pts)	Aid sensitivity (/10pts)		Transmissibility (/20pts)		Productive process efficiency (/25pts)	
Livestock	14	2.5	16.5	13.875	9.75	23.6	1.5	1.5	14.625	14.625
Without livestock	12.7	3.2	15.9	13.7	9.0	22.8	3.7	3.7	12.8	12.8

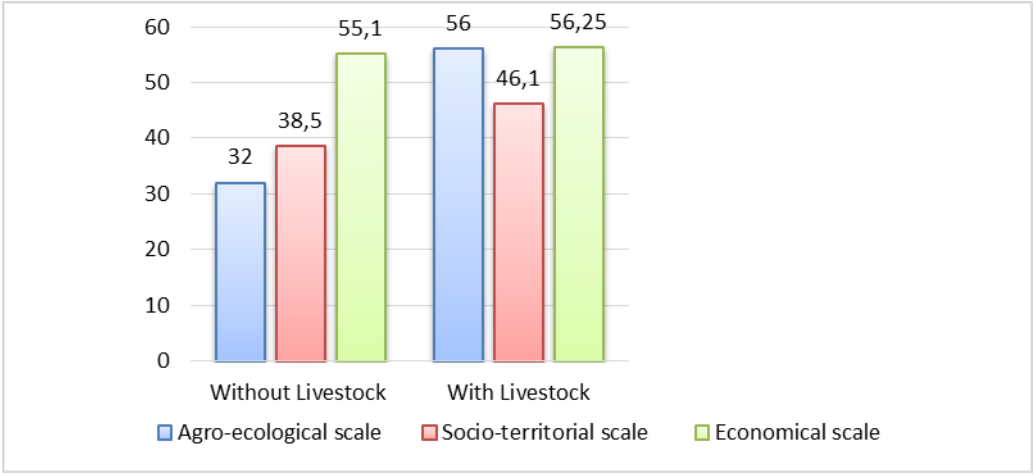

Figure 5. Representation of the averages of the three sustainability scales according to the farming method

The last component, "Efficiency", demonstrates the ability to generate value added by limiting operating costs and making the most of resources. Farmers practicing integrated agriculture have a better efficiency of the production process. In order to be able to confirm everything that is said previously, we decided to compare the sustainability of the farms surveyed in the Mornag zone according to the two farming ways: with livestock and without livestock farming (only cropping system).

Subsequently, we can see (Figure 5) that the group of farms that integrate livestock is more sustainable than the group of farms without livestock in all three scales. The biggest difference is found in the agro-ecological scale (32 points and 56 points), then the socio-territorial scale and, finally, the economic scale where the means of the two groups are similar.

CONCLUSIONS

From the analyses of the obtained results and the comparison between the means of the indicators of each component according to the farming mode, we were able to deduce that this factor considerably influences agricultural sustainability on both agro-ecological and socio-territorial scales. We can conclude that the group of farms that incorporate livestock has better averages in most indicators, in deed the association of crops with livestock within a production unit is considered an asset in the sustainability of a farm since it allows positive interactions and synergies between the different elements of the system. Indeed, in the mixed-crop-livestock system, the diversification of productions, crop rotation and the use of animal manures contribute to improving soil fertility.

In addition, animal husbandry allows income to be diversified and spread over time, thus ensuring certain stability in the economy. Some farmers even have a daily income from animal products such as eggs and chickens; others take advantage of some products for family consumption. The association of plant production with livestock makes it possible to promote the complementary relationships between cropping systems (fodder production, nitrogen fixation and soil fertility) and livestock systems (production of organic matter). Indeed, these complementarities make it possible to reduce the use of chemical fertilizers and concentrated feed. It also allows the use and exchange of by-products and allows them to be used as an input for the other system as animal feed and fertilizer, which saves resources but also preserves the soil resource. Crop-livestock integration is therefore a very important characteristic for a sustainable farming system.

Actions such as grazing animals inside or outside the farm, planting legumes or fodder crops have a positive impact on the environment, improve soil fertility and reduce inputs consumption. In addition, the integration of crops and livestock has a positive effect on the social and economic domains by creating jobs and increasing the level of productivity.

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NEW APPROACHES IN GROWING TECHNOLOGY OF *Valeriana officinalis* L. UNDER THE CONDITIONS OF CLIMATE CHANGE

SUMMARY

There is an acute shortage of soil moisture in critical periods of growth and development of *Valeriana officinalis* L. on the territory of Ukraine. Classical technologies previously used for growing crops do not allow to obtain consistently high yields of roots under the conditions of climate change. Therefore, in order to find new effective methods of valerian growing, research studies have been conducted to establish the impact of drip irrigation, mineral nutrition, sowing dates on productivity and phytosanitary condition of valerian crops in Ukraine. The moisture content of the root layer of the soil was maintained at the level of 85% of the lowest moisture content, when irrigation was used. Mineral fertilizers in the dose of $N_{90}P_{90}K_{90}$ were applied for the main tillage and $N_{30}P_{30}K_{30}$ for fertilization. Sowing was carried out in two terms (spring and winter). The yield of valerian depended only on the weather conditions of the year, when irrigation was not used. In the first year of research, the natural soil moisture was the highest among all years of research. The yield of valerian raw materials was 19.4 – 26.3 quintal/ha for spring sowing of the first year. For the second year of research – it was only 5.4 – 7.9 quintal/ha due to summer drought. For the third year no seedlings were obtained due to the spring drought. On average over three years, the use of drip irrigation in combination with mineral fertilizers provided 26.3 – 36.6 quintal/ ha yield of dry roots. Prolongation of vegetation during winter sowing, application of irrigation and mineral fertilizers (with their application in the dose of $N_{90}P_{90}K_{90}$ mainly) provided a yield of 36.8 quintal/ha. The highest yield of valerian roots was 42.2 quintal/ha obtained for winter sowing when irrigation was used in combination with the application of basic fertilizer $N_{90}P_{90}K_{90}$ + fertilization $N_{30}P_{30}K_{30}$. Under the conditions of drip irrigation dangerous pathogens for valerian were *Erysiphe*

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cichoracearum f. valerianae, *Ramularia valerianae*, *Uromyces valerianae*, *Septoria valerianae*, *Peronospora valerianae*, and also fungus of the genus *Fusarium* and viruses. Stable yields of valerian raw materials under the conditions of climate change were obtained with the use of drip irrigation and mineral fertilizers with the extension of the growing season of valerian due for winter sowing.

Keywords: *Valeriana officinalis* L., *drip, irrigation, yield, mineral fertilizers*

INTRODUCTION

Valeriana officinalis L. is one of the most widespread cultivated medicinal plants in Europe (Gholiloo et al., 2019; Gorban et al., 2004; Mishchenko et al., 2015; Romashchenko et al., 2015). According to the State Statistics Service of Ukraine, the in recent years area under this crop has decreased significantly. The decrease in the production of valerian raw materials is caused by a number of factors; climate change is one of the most important among them. Climate change includes long dry periods without rain. Such conditions, in combination with high average daily temperatures, lead to a significant decrease in the yield of agricultural crops, their infection by different pathogens, and often to the complete death of crops (Gholiloo et al., 2019; Filipovic and Kljajic, 2015; Mishchenko et al., 2019; Pryvedeniuk and Hlushchenko, 2020; El Bılalı et al., 2020). At the same time, the high quality raw materials, grown under the conditions of the Left-Bank Ukrainian Forest-Steppe zone, contain biologically active substances in concentration required by the Ukrainian and European standards. The shortage of valerian medicinal raw materials in the domestic pharmaceutical market is compensated by import of this plant (Pryvedeniuk et al., 2016). It is known that the medicinal plants raw materials quality can be significantly affected by viruses (Dunich and Mishchenko, 2015; Mishchenko et al., 2015).

According to the effect of mentioned factors on valerian growing, nowadays there is a necessity to improve technology of valerian production. One of the ways to solve this problem is improvement of water-nutrient regime. It could ensure the formation of high yields of appropriate quality for one year of cultivation. Extensive and successful implementation of drip irrigation in vegetable growing, potato farming and fruit harvesting show its effectiveness and prospects in medicinal plants cultivation (Filipovic, and Kljajic, 2015). Nowadays more attention is paid to study of the impacts of irrigation regimes on the spread and development of plant diseases. Irrigation affects several aspects of different cycles of plant diseases, such as sporulation, survival of the pathogen, their spread to new hosts, germination and infection. As follows, irrigation is the most important cultural practice that can protect from and reduce spread of plant diseases. The implement of drip irrigation was studied in order to solve mentioned problem. Research studies were held on valerian in the Research Station of Medicinal Plants IAE NAAS. Therefore, the aim of the study was to

develop new approaches in cultivation technology of *Valeriana officinalis* L. under the conditions of climate change and control of virus damage.

MATERIAL AND METHODS

In order to improve the technology of valerian growing by applying drip irrigation and mineral fertilizers field studies were held in crop rotations of the Research Station of Medicinal Plants IAE NAAS in Poltava region in Ukraine during three years. The soil of the research field - strong chernozem, low-humus, thickness of the humus horizon 87 - 100 cm, light particle size distribution. The reaction of the soil solution is mildly acidic, in terms of metabolic acidity the soil is characterized as moderately acidic.

The supply of soil with basic nutrients: easily hydrolyzed nitrogen - low, mobile phosphorus - very high, exchangeable potassium - increased. According to the amount of salts, the soil is unsalted. The lowest moisture content of 0 – 100 cm layer is 18.2%, for 0 – 50 cm layer is 17.5%. The density of the composition of 0 – 100 cm layer is 1.27 g/cm^3, for 0 – 50 cm layer it is 1.32 g/cm^3.

Methodological approaches used in Ukrainian practice and in medicinal plant growing have been used in the scientific research. In particular, the development of experimental schemes was performed according to the methods of Dospekhov with the peculiarities of drip irrigation mentioned in the method of Romashchenko M.I. Sampling of plants, biometric measurements and phenological observations were carried out considering the medicinal crops characteristics according to the methods of Brykin (Brykin, 1981; Dospekhov, 1985; Romashchenko, 2014;).

The study of the viral particles morphology was carried out using the method of transmission electron microscopy. The research was carried out using the equipment of the Center for Collective Use at the Danylo Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The viral preparation was applied to copper grids with a substrate film consisting of 0.2% solution of formvar on dichloroethane. Negative contrast of the drugs was performed with 2% aqueous solution of phosphoric-tungstic acid for 2 minutes (Saliga and Snitinsky, 1999).

The method of solid-phase enzyme-linked immunosorbent assay in the modification of a double sandwich (DAS-ELISA) was used to determine the presence of viral antigens. The analysis was performed using commercial test systems for *Potato virus M, S, X, Watermelon mosaic 2 virus, Potato virus Y, Cucumber mosaic virus, Tomato spotted wilt virus, and Alfalfa mosaic virus* manufactured by Loewe (Germany) in three repeats. A commercial viral preparation (Loewe, Germany) was used as a positive control in the reaction, healthy valerian leaves – as negative. The reaction results were recorded on a reader of Termo Labsystems Opsis MR (USA) with Dynex Revelation Quicklink software at wavelengths of 405/630 nm. Values that exceeded the negative control at least three times were considered valid (Crowher, 1995).

Statistical processing of EM and ELISA results was performed taking into account the standard deviation: $X = X \pm A\sigma$; $X = (X1 + X2 + \ldots + Xi) / i$; $A = |Xmax - X| = |Xmin - X|$, where X - is the significant value of the virion length / value of the optical density of the reader at a wavelength of 405 nm; X - is the arithmetic mean of the measured values of the virion length / value of the optical density; X1 … Xi; σ - is the standard deviation (Lakin, 1980).

The research field was planted by direct sowing in the soil of valerian seeds of the medicinal variety Ukraine with a rate of 6 kg/ha, seed wrapping depth – 1.0 cm, row spacing width - 60 cm. Winter sowing was carried out in the third decade of August, spring sowing – in the second decade of April. An irrigation pipeline with a diameter of 16 mm with integrated water outlets every 20 cm with a water flow rate of 1.2 dm^3/h was used for irrigation, one pipeline was used to moisten one row of plants. During the vegetation period, soil moisture in the layer of 0 – 40 cm was maintained at 80-85% of the lowest moisture content. NPK mineral fertilizer containing the active substance $N_{16}P_{16}K_{16}$ was used for the basic application and fertilization. Valerian medicinal crops were recorded in the first decade of October: first the aboveground mass of plants was mowed, then the roots with rhizomes were dug up, separated from the soil residues and the soil was washed from the freshly dug roots for 15 minutes. The roots were dried at temperature +35 ^oC to humidity 10 –12%.

RESULTS AND DISCUSSION

The obtained research results show that the productivity of valerian in variants without use of irrigation depended on weather conditions, in particular the presence of soil moisture in critical periods of growth and development of the crop. Therefore, for the first year of research soil moisture during the growing season of valerian spring sowing was the highest among three years of research. This moisture level provided optimal seedling formation and, consequently, the yield of dry roots with rhizomes at 19.4 – 26.3 quintals/ha without irrigation. For the second year of research, soil moisture was sufficient for the growth and development of valerian, but during June-August there was an acute lack of moisture, so the yield of roots was only 5.4 – 7.9 quintals/ha. The spring period of the third year of research was characterized by the absence of precipitation with high average daily temperatures; as a result valerian seedlings were not obtained at all. (Table. 1). For the spring sowing of valerian, the best conditions for plant growth and development was when irrigation was used together with mineral fertilizers. Therefore, on average over three years, under conditions of drip irrigation, the yield of roots with rhizomes was in range of 26.3 – 36.6 quintals/ha. When irrigation was used without mineral fertilizers the yield was 26.3 quintals/ha, exceeding the variant without irrigation and without fertilizers (control) by 13.9 quintals/ha or 112%. The efficiency of mineral fertilizers application in the presence of soil moisture increases rapidly, which is confirmed by the additional yield of roots of 6.5 quintals/ha when watering and basic

application of mineral fertilizers were applied compared to the variant with irrigation without fertilizers.

Table 1. Effect of soil water-nutrient regime on the yield of valerian dry roots with rhizomes during spring sowing.

Variants		Yield of dry roots, quintal/ha				% of control group
Factor A	Factor B	1st year	2nd year	3rd year	Average	
Without irrigation	Without fertilizers	19.4	5.4	-	12.4	100.0
	Basic application of $N_{90}P_{90}K_{90}$	21.8	7.2	-	14.5	116.9
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	26.3	7.9	-	17.1	137.9
Drip irrigation	Without fertilizers	26.5	28.4	24.1	26.3	212.0
	With basic application of $(NPK)_{90}$	31.6	36.2	30.7	32.8	268.5
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	36.3	39.4	34.1	36.6	295.2
$LSD_{0,5}$ main effects f. A		1.4	1.1	1.6	2.8	-
$LSD_{0,5}$ main effects f.		1.9	1.8	1.3	2.2	-
$LSD_{0,5}$ partial differences f.		2.5	2.0	2.9	4.4	-
$LSD_{0,5}$ partial differences f.		2.3	2.2	1.7	2.6	-

The highest yield of valerian roots 36.6 quintals/ ha during spring sowing was obtained when irrigation was used together with basic application of mineral fertilizers and additional fertilization, which exceeded the control by almost three times.

The study was provided by studying to predict the period of valerian winter sowing study under the conditions of drip irrigation. This sowing period provides an increase in the growing season of the crop from sowing to harvest and promotes the formation of higher yields compared to spring sowing. For winter sowing, the research plots were laid in the third decade of August. The autumn period of the first year of research was characterized by an acute shortage of soil moisture with high temperatures, as a result valerian seedlings was not obtained without irrigation. When irrigation was used, the phase of full germination of valerian was observed on the 8th day after sowing, before the onset of persistent frosts, the plants formed 3-4 true leaves, which contributed to successful overwintering. The autumn period of the second research year was favorable for obtaining seedlings and their growth and development without irrigation, the yield of valerian roots at the end of the growing season was 17.3 – 20.3 quintals/ha (Table. 2).

Table 2. Effect of soil water-nutrient regime on the yield of valerian dry roots with rhizomes during winter sowing

Variants		Yield of dry roots, quintal/ha			% of control group
Factor A	Factor A	1st year	2nd year	Average	
Without irrigation	Without fertilizers	-	17.3	17.3	100.0
	With basic application of $N_{90}P_{90}K_{90}$	-	19.2	19.2	111.0
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	-	20.3	20.3	117.3
Drip irrigation	Without fertilizers	31.6	32.1	31.9	184.4
	With basic application of $N_{90}P_{90}K_{90}$	38.7	34.9	36.8	212.7
	With basic application of $N_{90}P_{90}K_{90}$ + addition of $N_{30}P_{30}K_{30}$.	44.5	39.8	42.2	243.9
$LSD_{0,5}$ main effects f. A		0.9	1.3	2.4	-
$LSD_{0,5}$ main effects f. B		1.4	1.0	2.2	-
$LSD_{0,5}$ partial differences f. A		1.5	2.3	3.6	-
$LSD_{0,5}$ partial differences f. B		1.7	1.3	2.7	-

Under the conditions of drip irrigation, for winter sowing, the yield of valerian exceeded the variant without the use of irrigation by 14.6 quintals/ha. The basic fertilizer application under irrigation conditions increased the root yield by 4.9 quintals/ha compared to the variant without fertilizers when irrigation was used.

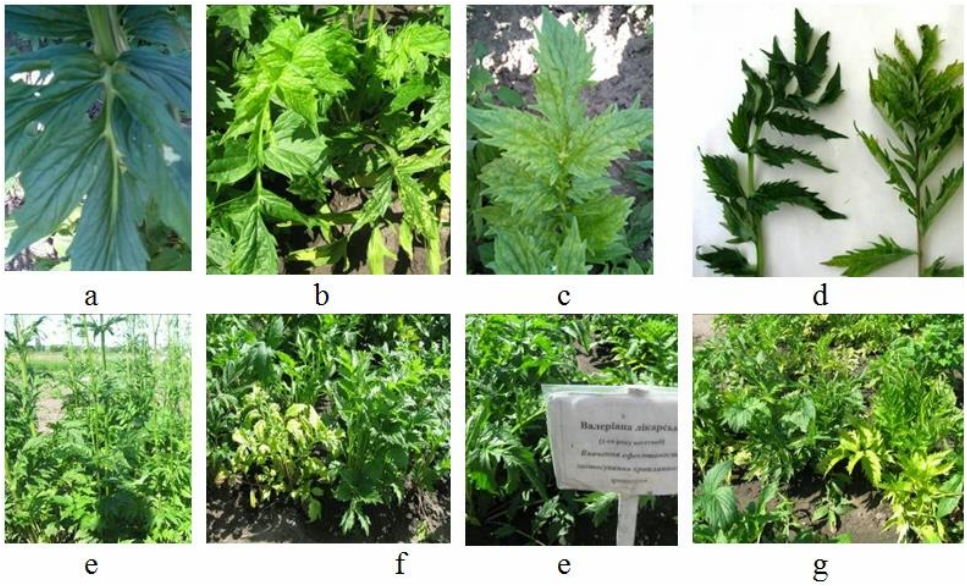


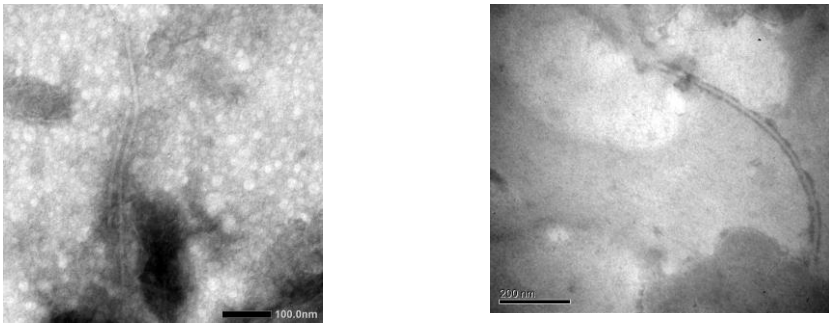
Fig. 1. *Valeriana officinalis* L. with symptoms of viral disease (b, c, d, f, g) and healthy (a, e); d - healthy on the left

The best conditions for the valerian growing and development were observed when irrigation was used together with basic fertilization and additional

fertilization, the yield of dried roots with rhizomes under such conditions was 42.2 quintals/ha, exceeding the control by 24.9 quintals/ha.

Under the conditions of drip irrigation, fungal and viral diseases are dangerous pathogens for valerian. Viruses are insufficiently studied both in Ukraine and in the world in general. Our previous studies in 2006-2009 showed that under traditional growing conditions 15-20% of *Valeriana officinalis* L. plants had symptoms of a viral disease (yellow mosaic, stunting and peduncle reduction), in which filamentous viral particles 530 ± 20 × 10-12 nm were detected (Mishchenko et al., 2009). Under conditions of drip irrigation (since 2013), valerian plants with symptoms of stunting and yellow ringspot on the leaves were also detected, but their number was lower compared to traditional growing conditions of this crop and was 2-5% (Fig. 1).

Our attention was drawn to new symptoms for this culture – ring spot on the leaves (Fig. 1 b, c). We have detected filamentous viral particles with a size of 550-600 × 11-12 nm (Fig. 2 a) and 750-800 × 11-12 nm (Fig. 2 b) in valerian leaves with this symptom by TEM.



a b

Fig.2. Electron microscopy picture of viral particles detected in the leaves of *Valeriana officinalis:* a – 550-600 nm, b – 750-800 nm

The morphology of the revealed virions is similar to carlaviruses and potexviruses (Fig. 2 a), and potyviruses (Fig. 2b), respectively (King et al., 2012).

ELISA was used to identify these viruses. For this purpose, test systems for several viruses were selected based on literature data and morphology of the studied virions. It should be mentioned that the identification of the viruses infecting valerian is quite difficult, as they are polyphagous and infect a wide range of different plant species. So, most likely, they are transmitted to valerian from near growing wild and agricultural crops. Therefore, to identify virions with a size of 550-600×11-12 nm testing was performed using test systems for viruses from genus *Potexvirus* and *Carlavirus*, which are widespread in the agrocenoses of our country - *Potato virus M, S, X*. According to the literature (King et al., 2012) valerian is susceptible to infection with the filamentous *Watermelon mosaic 2 virus*, which belongs to the genus *Potyvirus* of the family *Potyviridae*. Therefore, in order to identify the virus with a size of 750-800 × 11-12 nm, test

systems were used for potyviruses *Watermelon mosaic 2 virus* and *Potato virus Y*. In addition, testing was performed for the presence of spherical viruses *Cucumber mosaic virus*, *Tomato spotted wilt virus* and bacilliform *Alfalfa mosaic virus*, which are registered in Bulgaria and Italy on valerian plants and circulate in Ukraine on other crops. ELISA results showed absence of antigens of all of the mentioned above viruses in the valerian plants leaves (Fig. 3).

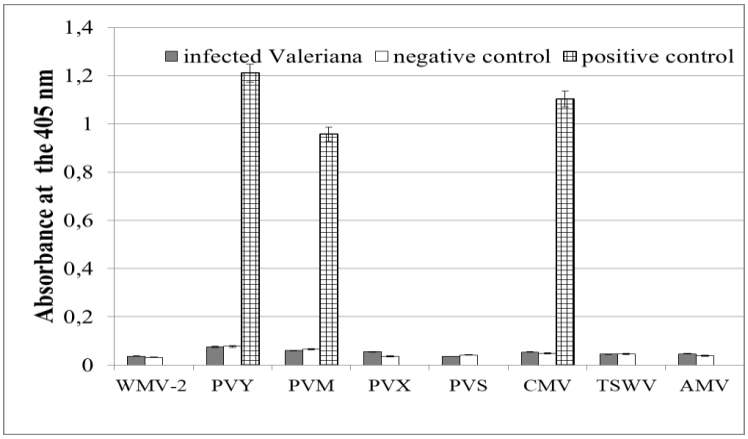


Fig. 3 Content of viruses` antigens in leaves of infected *Valeriana officinalis*

As mentioned above, the identification of viruses infecting valerian is difficult. This also applies to other medicinal plants, such as *Echinacea purpurea*, *Panax ginseng* and others. The literature data suggests that they do not have "own" highly specialized viruses and are affected by polyphagous viruses. This was the case with mint plants, but the development of modern molecular studies of plant viruses has allowed scientists to determine that peppermint plants still have their own highly specialized viruses, which were included in ICTV (Tzanetakis et al., 2005a, b). Therefore, we can assume that the viruses we have detected are also not yet described and are the new valerian viruses. At the time of this manuscript writing, the results of molecular research conducted by Japanese researchers (Uehara-Ichiki et al., 2020) on the first detection of two new carlaviruses on *Valeriana fauriei* plants - *Gaillardia latent virus* (GaILV), *Ligustrum necrotic ringspot virus* (LNRSV). Therefore, our further studies of viruses that infect valerian in Ukraine will include testing them for GaILV and LNRSV presence.

CONCLUSIONS

The obtained research results prove that the cultivation of valerian under the conditions of the Left-Bank Forest-Steppe zone of Ukraine without the use of irrigation is risky. Due to the deficit of soil moisture in the critical periods of growth and development of the crop, a low yield of valerian is formed in the range of 12.4 – 17.1 quintals/ha for spring sowing and 17.3 – 20.2 quintals/ha for

winter sowing. For some years, due to drought, it is impossible to get seedlings of valerian. The application of drip irrigation allows to obtain guaranteed yields of dry roots with rhizomes of valerian in the range of 26.3 – 36.6 quintals/ ha for spring sowing and 31.9 – 42.2 quintals/ha for winter sowing. Due to the lack of soil moisture, the use of mineral fertilizers without irrigation is ineffective. Under the conditions of drip irrigation, the basic application of mineral fertilizers with additional fertilization increased the yield of raw materials by 10.3 quintals/ha compared to the variants without fertilizers.

The highest yield of valerian 42.2 quintals/ha was obtained for winter sowing when drip irrigation was used together with the basic application of mineral fertilizers and additional fertilization, which is explained by the creation of the most favorable water and nutrient conditions for growth and development of the crop. Drip irrigation together with mineral fertilizers and extension of the growing season for winter sowing of valerian provides stable yields of raw materials under the conditions of climate change

Knowing the pathogen is important to determine the type of irrigation, frequency and volume of water used to control one specific plant disease. It could be a key factor in achieving high yields. Viruses, transmitting with their vectors, can be controlled by sprinkle irrigation, which disrupts the contact of the insect with the plant» (Café-Filho and Lopes, 2018). Therefore, our data on a smaller number of virus-infected valerian plants under drip irrigation could be recommended for further use for valerian cultivation.

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THE INFLUENCE OF UNCONVENTIONAL MINERAL FERTILIZERS BASED ON THE PROCESSING OF K-MG ORES ON YIELD AND QUALITY OF SEED POTATO, AS WELL AS SOIL FERTILITY PARAMETERS

SUMMARY

The results of the field study with traditional mineral fertilizers and unconventional one, based on the processing of K-Mg ores, on seed potato planting are presented in this paper. The efficiency and aftereffect of potassium fertilizers (KCl and the processing product from clay-salt sludge) on the NP background were studied. The technology for the production of new fertilizer from clay-salt waste (sludge) remaining as a result of potassium fertilizers production from natural K-Mg ores, was elaborated in Mining Institute, the division of Perm Federal Research Center. The resulting product, containing K, Ca, Mg and trace elements, which has the properties of fertilizer and ameliorant, has received the name the cinder of clay-salt sludge. The main processes in the technology are waste enrichment and subsequent high-temperature calcination of the enriched concentrate. As a result, the product, with the properties of multiple slow-release fertilizer was formed on a chloride-free basis. It is undesirable to use for potato fertilizers containing chlorine, so it is useful to expand the range of potassium fertilizers. The field trials with cinder were fulfilled on the experimental plots of Perm Agricultural Scientific Research Institute, the division of Perm Federal Research Center, in 2018-2020 years. The experimental scheme included treatments: control (without fertilizers), NP - background; NP + KCl and NP + cinder. Potassium fertilizers were used in autumn (basic application) and in

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spring (pre-planting application). No fertilizers were used in 2020. The influence of mineral fertilizers on yield and quality of seed potato, as well as soil fertility parameters were studied. The obtained results showed that the direct effect of unconventional fertilizer (cinder) with its pre-planting application was approximately equal to the effect of traditional potassium chloride fertilization, provided that the rates in the active substance were equal (yield in 2019 – 46.0 and 47.1 t^{-1} ha, respectively). The use of cinder did not have a significant effect on the quality indicators of potato tubers both directly in the year of fertilization and after the storage period. Cinder, in contrast to the standard KCl fertilizer, had a positive effect on the content of exchangeable potassium in the soil. Research with unconventional mineral fertilizers based on K-Mg ore processing wastes should be continued with an emphasis on the study of long-term action as a complex ameliorant.

Keywords: Seed potato, mineral fertilizers, K-Mg ores, potassium, yield, soil fertility

INTRODUCTION

Potato *(Solanum tuberosum L.)* is one of the main food crops in the world. Total area occupied by potato in the world according to FAO, in 2015 was about 19 million hectares (FAOSTAT, 2015). The area occupied by potato in Russia, is about 2 million hectares. It is the first place in Europe and the second place in the world after China.

The total volume of world potato production in 2019 amounted to 376.8 million tons, in Russia - 29.8 million tons, it is third place in the world after China – 89 million tons and India – 45.3 million tons. At the same time, the average yield of this crop in the country, about 15 t ha^{-1}, remains one of the lowest (Zhevora, *et. al.*, 2019). The main reasons for this include the low quality of planting material, significant losses during the growing season as a result of damage by numerous pests and diseases, as well as due to high spread of weeds. The consequences of abiotic stresses, such as droughts, unfavorable physical and agrochemical properties of soils, and unbalanced mineral nutrition, make a significant contribution to the decrease of potential yields (Korshunov, *et. al.*, 2003, Zhevora, *et. al.* 2017).

Potato does not tolerate acidic soils with low organic matter content. Most of the arable land in the Non-Black Earth Zone of Russia is occupied by acidic sod-podzolic soils. It is necessary to fulfill set of measures to increase their fertility, improve their agrochemical and agrophysical properties in order to use these soils for potato requirements.

The most important features of this culture include increased requirements for nutrients, especially potassium, as well as a long period of nutrition. The maximum input of nitrogen and phosphorus by plants usually occurs before flowering, while the supply of potassium continues until the full physiological maturity of tubers. Potassium stimulates the synthesis of sugars and high molecular weight carbohydrates - starch, cellulose, pectin substances, xylans,

both in leaves and in potato tubers (Korshunov, *et. al.*, 2003). The results of numerous studies prove that the greatest return from the use of nitrogen and phosphorus fertilizers can be achieved only on the background of a sufficient supply of potassium. This trend is well traced not only for crops that consume large amounts of potassium (potato, root crops), but also for cereals and forage crops (Mikhailova *et. al*., 2013, Rajičić V., *et. al*., 2019, Yamaltdinova V.R. *et. al*., 2019, Rajičić V., *et. al*., 2020).

Potato is responsive to fertilization, primarily potassium. The main type of potassium fertilizer used in agriculture is potassium chloride. However, for potato, it is undesirable to use fertilizers containing chlorine, since carbohydrate metabolism is disturbed under the influence of it during the formation of the crop. Therefore, it is necessary to expand the range of other forms of potash fertilizers.

Many researchers (Smetannikov *et. al*, 2017) consider that in some cases it is advisable to use complex fertilizers, which include macro- and microelements, as well as ameliorating minerals: dolomite, calcium carbonates and sulfates. Potato needs a neutral or slightly acidic soil, so the use of ameliorants has a positive effect on its productivity. Potato is often planted on peaty soils, where there is a lack of trace elements, primarily copper. Copper is also a part of many fungicides, therefore, a full supply of potato plants with copper can play a role in the prevention of fungal diseases. Potato plants also need other trace elements, the main of which are Zn, B, Mo. The use of complex fertilizers, which include macronutrients, as well as copper, iron, zinc, boron, molybdenum, according to Sharipova *et. al*. (2016), reduces the pesticide load on plants by 30 percent, increases yields by 15-30 percent.

With the autumn application of fertilizers before fall plowing, nutrient losses often occur during the autumn-winter period, as well as during the spring period as a result of snow melting. Extremely large losses are observed in regions with a flushing water regime, as well as in the case of the use of water soluble fertilizers, for example, potassium chloride. In this connection, a search is being made for ways to reduce these losses. One of the methods is the use of fertilizers with reduced solubility (slow- release). Other ways to slow down the input of nutrients to the soil are granulation and encapsulation, but these techniques slow down dissolution to a small extent (Krutko and Shevchuk, 2011, Gurbanova *et. al.*, 2017).

Sometimes, the products of sludge and chemical reagents sintering used as slow- release fertilizers (Hu et. al., 2018), as well as the product of the merlinoite synthesis (K_5Ca_2 ($Si_{23}A_{l9}$) O_{64} x $24H_2O$) from coal and other products (Li, *et. al*., 2014, Ruthrof, *et. al.*, 2018).

An accompanying, but essential role play the possibility of waste disposal in the production of potassium fertilizers. One of the largest deposits of potassium, magnesium and sodium salts, located in Russia - Verhnekamskoe, contains a third of the world's reserves, which are mainly used for the potassium fertilizers production. This deposit is located in the north of Perm Region (Solikamsk). Mining wastes in interaction with natural and technogenic

compounds form organic-mineral complexes that represent a great environmental hazard (Bachurin *et. al*., 2014). It is extremely important that the reserves of clay-salt sludge in the sludge storage facilities of "Uralkaly" company amount more than 50 million tons. In addition, the annual output of sludge from six ore processing plants is more than two million tons (Onosov and Smetannikov, 2014).

Studies of waste products from the mining industry have shown that their complex utilization, in addition to environmental value, can bring significant profits. Sludge is a suspension, which contains 30 percent water-insoluble residue (hereinafter WIR), up to 30 percent residual K and Na chlorides, and about 40 percent H_2O. In turn, WIR contains the following minerals: quartz, potassium feldspar (FS) - a source of potassium; calcium sulfates (gypsum and anhydrite), dolomite, which are ameliorant minerals, then, sulfides Cu, Zn, etc., which can be positioned as micronutrient fertilizers, the content of the latter reaches more than 600 g t^{-1}.

In addition, WIR contains precious metals - Au, Ag and Pt, the total content of them reaches more than 40 g t^{-1}. This is followed by the rare earth elements Nb, Ta, Zr, W, among which cerium predominates, the content of which reaches 174 ppm. It is believed that cerium and its compounds are elements that may be of interest for inhalation therapy (Tuev and Mishlanov, 2008).

Residual chlorides and technogenic elements interfere the use of sludge as fertilizers. Residual reagents (amines, polyacrylamide, carbamides, neonol, etc.) used to enrich potash ores in the production of fertilizers Together with the sludge, they enter the places of their storage - sludge storage and become ecopollutants (Smetannikov, *et. al.*, 2018)

Scientists and specialists from the Mining Institute of the Ural Branch Russian Academy of Sciences have developed a technology for enrichment and processing of sludge, which excludes technogenic components from their content, as well as the transformation (destruction) of residual potassium and sodium chlorides, in order to ensure the incorporation of conversed products into secondary potassium minerals and ameliorant minerals (Smetannikov, *et. al*, 2017). The main method was high-temperature roasting, which made it possible to remove the residual amount of organic reagents, as well as to convert sulfides into oxides, which are sorbed on matrix minerals. Thus, it is possible to use the product as complex fertilizer with trace elements owing their slow release. The resulting chloride-free product was named cinder of clay-salt sludge, hereinafter cinder. (Smetannikov, *et. al.*, 2013, Onosov and Smetannikov, 2014). Preliminary studies carried out in 2014-2016 by scientists of the Mining Institute and Perm Agricultural Research Institute of the Ural Branch Russian Academy of Sciences have shown that the cinder effect for grain crops is approximately equal the one of traditional potassium fertilizer, provided that the doses in the active substance are also equal (Smetannikov, *et. al.*, 2017). The essential advantages of this type of fertilizer are its complex nature (the presence of K, Ca, Mg and trace

elements), the practical absence of chlorides, the ability to improve not only the agrochemical, but also the agrophysical properties of the soil.

Studies continued in 2018 on seed potato plots showed that complex slow-release fertilizers were not inferior to traditional (potassium chloride) fertilizers concerning their impact to soil agrochemical properties, potato yield and quality of tubers. The content of heavy metals in potato tubers did not exceed the MPC when the full mineral fertilizer was applied at the recommended rate and was at the level of the background and control variants. Similar results were obtained for the content of heavy metals and microelements in the soil. Their content was almost the same in the treatments with cinder and potassium chloride. (Smetannikov, *et. al.*, 2019).

Unlike other types of slow-release fertilizers, for the production of which additional technological operations are required (synthesis of organic compounds - complexones, etc.), the studied fertilizer form - the cinder contains a ready-made mineral matrix. The minerals included in its composition can serve as a basis or a matrix for macro- and microelements, which allows to add an almost unlimited set of components to it (as well as plant pesticides, growth stimulants and bacterial preparations). Thus, a conceptual model for the creation of new types of mineral fertilizers with desired properties is being formed, which makes it possible to use them on different soils and climatic conditions and for crops with different requirements for mineral nutrition (Smetannikov, *et. al.*, 2019).

In 2017 the research program for the implementation of this concept has been launched in Perm Federal Research Center of UB RAS which included the Mining Institute and PARI as divisions. The general aim is to develop the technology for obtaining complex fertilizers from waste products of K-Mg ores and their use for seed potato and other crops. The research objectives aim in 2020 is to determine the effect of cinder as a complex ameliorant on the yield and yield quality of seed potatoes, as well as on the agrochemical indicators of sod-podzolic soil. According to agronomic practice for most of agricultural crops, PK fertilizers are applied in autumn, under the fall plowing. However, under unfavorable conditions, fertilization may be postponed to the spring before planting potato. Therefore, the auxiliary task was to determine the influence of fertilization timing on potato yield and tubers quality.

MATERIAL AND METHODS

The experimental work was fulfilled in 2018-2020 in the laboratory of technological mineralogy of Mining Institute and on the experimental farm of Perm Agricultural Research Institute situated in the suburbs of the city of Perm - center of Perm Region in Russia. The experimental site is 58° 0.615' 0" N 56° 14.051' 0" E .

The climate of Perm Region is continental with long cold and snowy winters and warm short summers. The Ural Mountains play an important role in the formation of the climate, which retain the moist air masses coming from the Atlantic Ocean. The average monthly air temperature of the coldest month

(January) is –15.7°C, the warmest month of July is +18.2°C. Average (over the last 70 years) annual air temperature + 2.4°C, for the growing season (May-September) +13.9°C.

The first autumn frosts are observed in 1-2 decades of September, the last spring frosts occur in the third decade of May - the first decade of June. The duration of the frost-free period on average in the region is 80-120 days. Annual precipitation in the region is 450-600 mm, average amount for the growing season is 320-450 mm. The formation of stable snow cover occurs at the end of the third decade of October - in the first decade of November, the height of the snow cover reaches 50-60 cm, the melting of the snow cover occurs in the third decade of April.

The objects of the study were potato plants of the variety "Nevsky" and sod-podzolic heavy loam soil (Umbric Albeluvisols Abruptic) with following agrochemical parameters: pH_{KCl}– 5.4, content of mineral N – 30 mg kg^{-1}, P_2O_5 – 410 mg kg^{-1}, K_2O – 100 mg kg-1, Ca – 11,3 mmol 100 g^{-1} , Mg – 3,3 mmol 100 g^{-1} (2018, before experiment foundation).

The subjects – were the traditional potassium fertilizer – potassium chloride (KCl) and cinder. The technology of obtaining cinder from clay-salt sludge includes following stages: hydrocycloning, filtration, granulation, drying, high temperature firing and protected by the patent RF № 2497961 from 10.11.2013. The used cinder was obtained at a firing temperature of 900°C, the optimum temperature for removing the residual amount of organic reagents and chlorine as well as to convert sulfides into oxides (Onosov and Smetannikov, 2014).

The main chemical compounds in cinder are following (mass percent): SiO_2 - 29,34; Al_2O_3 -6,03; Fe_2O_3 -6,48; CaO -13,9; MgO - 12,44; Na_2O - 5,18; K_2O - 4,84; P_2O_5 -1,38; S – 0,19; trace elements content (mg kg^{-1}): Ni - 320,8; Cu -156,5; Zn - 153,2; As - 20,1; Mo- 3,22.

Analyses were fulfilled in Analytical Certificate Testing Center (Moscow) on atomic emission spectrometer with inductive-chemical plasma Optima-4300 DV and mass spectrometer with inductive-structure plasma Elan-6100.

The study of direct action took place in 2019, aftereffects - in 2020. The applied fertilizers: 1) NP commercial fertilizer mixture with N content 22.43 percent , P_2O_5 – 21.12%, 2) Potassium chloride – K_2O 59.29 percent 3) Cinder (C3) - K_2O 4,84 percent. Fertilizers rates (active ingredient): $N_{90}P_{90}$ and $N_{90}P_{90}K_{90}$. Fertilization timing in 2018-2019 - autumn (basic application–BA and spring (pre-planting application–PA). No fertilizers were used in 2020. The cultural practice is usual for potato in Perm Region excluding the studied elements.

Experimental scheme

1.Control (no fertilizers);

2.NP- background (pre-planting application)

3.Background + KCl (basic application)

4.Background + C3 (BA)
5.Background + KCl (PA)
6.Background + C3 (PA)

Treatment placing is systematic, each treatment has three replications on field area. Plot area 32.8 m^2.

Soil and plant chemical analyses were fulfilled in analytical laboratory of Perm Agricultural Research Institute, according valid national standards. The obtained data were statistically processed by analysis of variance (using least significant difference - LSD).

RESULTS AND DISCUSSION

The growing season of 2019 was characterized by slightly elevated temperature and moisture deficit in the first half, by sharp heat deficiency and excessive rainfall - in the second. The average season temperature was +13.5°C, which is 0.4°C, lower than multiyear average, precipitation sum was 535 mm (160 percent from multiyear average – 335 mm). Extremely wet and cold weather conditions were in August, rainfall amount was 233 mm (306 percent). The conditions for tuberization process and harvesting were unfavorable.

The growing season of 2020 was drier and hotter. The average season temperature was +14.8°C, precipitation sum was 332 mm (99 percent). In July, when the budding and flowering phases were observed on the plants, hot weather was noted, which, with a significant, almost 2 times, deficiency in rainfall amount, had a significant negative effect on potato productivity. The influence of cinder and traditional mineral fertilizers application (2019) on and their aftereffect (2020) is presented in the Table 1.

Table 1. Cinder and traditional mineral fertilizers impact on potato yield (Nevsky variety)

Treatments	2019			2020		
	Yield, t ha^{-1}	Supplements		Yield, t ha^{-1}	Supplements	
		against control, t ha^{-1}	to the background, t ha^{-1}		against control, t ha^{-1}	to the background, t ha^{-1}
Control (no fertilizers)	36,2	–	–	8,59	–	–
NP- background	43,8	7,6	–	12,06	3,47	–
NP + KCl (BA)	44,9	8,7	1,1	13,77	5,18	1,71
NP + C3 (BA)	37,8	1,6	-6,0	9,13	0,54	-2,93
NP + KCl (PA)	47,1	10,9	3,3	13,32	4,73	1,25
NP + C3 (PA)	46,0	9,8	2,2	10,18	1,59	-1,89
LSD_{05}		6,3			2,21	

The overall yield in 2019 (Table 1) was significantly higher compared with 2020, because of fertilizer effect and weather conditions. The growing season of 2019 was characterized by excessive amount of precipitation, and 2020 - by dry weather conditions. All studied treatments with fertilization provided an increase in yield compared to the control, but the magnitude of the increase varied

significantly among treatments and by years of observation as well. The supplements ranged from 2.9 to 12 t/ ha^{-1}.

The only treatment with fertilizers use (cinder in the fall) did not provide a significant increase in potato yield. The increase was within the experimental error. The largest increments from 11 to 12 t^{-1} were provided by the spring application of potassium fertilizers on the background of NP. The effect of cinder was approximately equal to traditional potassium chloride. It should be noted that the increase in yield was obtained mainly due to nitrogen and phosphorus fertilizers. The autumn application of potassium fertilizers did not provide an increase in yield compared to the background. Perhaps, it may be connected with potassium leaching in the extremely rainy autumn in 2018.

The results of 2019 can be explained by unfavorable weather conditions during the tuberization period, when the temperature was below optimal, and precipitation amount was significantly higher compared with multiyear average. It is the very period when potassium is most needed for potato, which absorption into plants under these conditions is difficult. This is confirmed by the results of research by Mikhailova *et. al.,* (2013), who argues that in a year with excessive moisture, an increase in yield can be obtained only due to nitrogen and phosphorus. Some authors (Pukhalskaya *et al*., 2009, Grzebisz *et al*., 2020) considered that potassium improves plant nutrition with nitrogen, while not independently causing an increase of yield.

In 2020, the effectiveness of fertilizers application (aftereffect) was low. The use of cinder did not provide an increase in yield against the control, and compared to the background there was a slight decrease in the supplements value. The observed deviations were within the experimental error or close in magnitude to $LSD_{05.}$

Table 2. Influence of the action (2019) and aftereffect (2020) of cinder on the yield structure of potato.

Treatments	Mass of tubers, g per plant		Number of tubers per plant		Average tuber mass, g		Seed fraction, percent	
	2019	2020	2019	2020	2019	2020	2019	2020
Control (no fertilizers)	1074,0	301,6	19,2	11,8	56,3	25,4	80,3	69,8
NP- background	1200,7	444,9	17,5	14,5	69,0	33,6	70,5	68,7
NP + KCl (BA)	1276,0	486,3	19,2	8,2	66,4	37,9	64,4	61,5
NP + C3 (BA)	1120,7	318,5	18,3	13,5	61,5	33,5	76,4	59,8
NP + KCl (PA)	1334,7	411,6	19,3	14,0	69,0	30,1	68,1	55,5
NP + C3 (PA)	1284,9	317,4	19,3	8,4	66,9	37,1	70,4	72,6
LSD_{05}	$F_ф<F_т$	88,1	$F_ф<F_т$	3,6	9,6	5,9	11,8	10,7

The low potato yield in 2020 is confirmed by data on the structure of potato yield (Table 2). The mass of tubers from one plant this year was on average 3-3.5 times less compared with 2019. In 2019, the mass of tubers varied within 1074-1334 g per plant without significant differences between treatments

(Ff <Ft). In 2020, the use of NP fertilizers and complete mineral fertilization in the traditional version with potassium chloride provided a significant increase of tuber mass from one plant, due to increase both the number of tubers and the average mass of one tuber. The smallest mass of tubers was obtained in treatments with cinder application and in the control treatment - 301-318 g per plant.

In all studied treatments with the use of fertilizers, the average mass of the tuber was significantly higher compared with the control, without significant differences depending on the types and combinations of fertilizers. In general, the yield raise was due to an increase in the weight of one tuber, the number of tubers per plant remained approximately at the same level with the control. It may be considered the variety peculiarity. The same results were obtained by other researches (López-Martín, *et al*., 2018). The content of the seed fraction in the yield slightly decreased in comparison with the control because of the increase of large tubers number. In the treatment without fertilizers, the share of the seed fraction reached 80 percent in 2019 and was about 70 percent in 2020, which is, respectively, high and satisfactory results for seed potato plantings (Torikov *et. al.*, 2011, Anisimov and Zebrin, 2018, Zhevora, *et. al.*, 2019) In the treatments with fertilizers seed fraction content varied from 55 to 76 percent, what was, as a rule, within the experimental error.

Table 3. Influence of the action (2019) and aftereffect (2020) of cinder on the yield quality parameters of potato.

Treatments	Dry matter, %		Nitrates, mg kg $^{-1}$		Starch, %	
	2019	2020	2019	2020	2019	2020
Control (no fertilizers)	15,39	19,80	18,9	93,75	10,6	12,88
NP- background	16,61	18,90	18,1	82,10	11,1	12,04
NP + KCl (BA)	16,30	20,83	17,7	77,40	10,7	13,90
NP + C3 (BA)	14,85	20,41	19,1	65,50	10,7	12,88
NP + KCl (PA)	15,33	21,19	19,4	76,22	9,8	13,84
NP + C3 (PA)	14,43	19,29	19,2	91,20	9,5	12,98
LSD_{05}	0,94	0,58	$F_ф<F_т$	11,34	0,6	0,41

The use of mineral fertilizers types in this experiment did not have a significant effect on the quality parameters of potato tubers yield (Table 3). The dry matter content varied according to the within 1-1.2% in 2019, which slightly exceeds the value of HCP05 - 0.94. Such an excess does not play a significant role in practical terms. The use of cinder reduced the percentage of dry matter in potato tubers compared to NP background, but this trend was not confirmed in 2020. It should be noted that in 2020 the dry matter content in potato tubers was on average 4-5% higher compared to 2019, which is explained by the low potato yield this year because of dry weather conditions during the period of crop formation. This trend was noted by other researchers (Neronova and Golubeva, 2012).

The nitrate content in 2019 in all the studied treatments was practically the same, the differences were within the experimental error, the starch content also did not have significant deviations from the control. The similar situation with the starch content was noted in 2020. Conflicting data have been obtained that year for nitrates content, so additional study is required. In the variants with NP application the content of nitrates significantly decreased, which contradicts the data of other research works (Pukhalskaya *et. al.*,2009, Mikhailova *et. al.*, 2013). At the same time, the content of nitrates in 2020 was 4-5 times higher compared with the corresponding indicators in 2019, which can be explained by the low yield of potato this year connected with a lack of moisture.

The starch content decreased in treatments with spring application of both potassium fertilizers by one percent approximately compared with the control (2019), but this trend did not confirm next year. The slight aftereffect of KCl use was noted. In our previous research the quality of potato tubers significantly decreased, but at higher doses of fertilizers – $N_{150}P_{150}$ K_{150} (Korlyakov, *et. al.*, 2018).

An important task in improving the technology of potato production is to preserve the harvest. It is known that cultivation technology, as well as weather conditions, can affect the safety of tubers in different ways. Therefore, some of the tubers were laid according to the treatments for storage in September 2019, after harvesting. In May 2020, the weight loss was taken into account in comparison with the weight before storing, and the quality indicators of potato after storage were studied (Table 4).

Table 4. Impact of fertilizers on preservation degree of potato yield and its quality parameters (2020).

Treatments	Dry matter, percent		Starch, percent		Percent of the original sample weight, preserved by planting time
	in spring	percent to autumn indicators	in spring	percent to autumn indicators	
Control (no fertilizers)	15,89	103,2	12,91	121,8	60,1
NP - background	14,80	89,1	12,56	113,2	65,0
NP + KCl (BA)	14,39	88,3	11,75	109,8	67,5
NP + C3 (BA)	13,27	89,4	11,60	108,4	58,7
NP + KCl (PA)	14,66	95,6	12,52	127,8	63,5
NP + C3 (PA)	13,44	93,1	12,44	130,9	68,0
LSD_{05}	1,1		0,33		$F_т > F_ф$

In the treatment without fertilizers, there was a slight increase in the dry matter content - by 3.2%, but the difference was insignificant, most likely within the observation error. Therefore, it can be assumed that in this variant the dry matter content during the storage period was stable, without significant changes.

The dry matter content decreased in spring in all studied variants with fertilizers compared with autumn indicators. The negative effect of the use of mineral fertilizers on the dry matter content preservation of potato during storage was also noted by Sabirov and Sabirova, 2012.

The picture with the content and preservation of starch was not so obvious: in the variants with autumn application of potassium fertilizers (KCl and cinder), the starch content was significantly lower compared with the control - by more than one percent on average, in the treatments with the spring application of potassium fertilizers it was slightly inferior to the control. At the same time, there was no decrease in the starch content during the storage period; on the contrary, it increased in all variants of the experiment. The most significant growth by 20-30 percent was observed in treatments with a higher initial starch content.

The total mass of the samples during storage decreased by 32-42 percent, with insignificant variation in the experimental variants, the difference between the variants was within the experimental error. Thus, the introduction of various types of fertilizers did not have a significant effect on the preservation of potato during storage. The tendency of starch content raising at the end of the storage period was found, this trend needs to be investigated in further research.

Table 5. Impact of mineral fertilizers application on agrochemical parameters of sod-podzolic soil.

Treatments	pH_{KCl}		Ca		Mg		Nmin		P_2O_5		K_2O	
			mmol 100 g^{-1}				mg kg^{-1}					
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control (no fertilizers)	4,74	4,63	10,7	11,3	3,6	2,1	26	21	362	294	116	115
NP - background	4,52	4,66	12,1	11,6	3,3	1,7	34	16	432	277	108	107
NP + KCl (BA)	4,57	4,66	11,6	10,5	3,5	2,0	30	17	376	327	143	113
NP + C3 (BA)	4,79	4,83	11,1	10,6	4,0	1,3	30	17	384	365	149	183
NP + KCl (PA)	4,54	4,58	10,6	11,1	4,3	2,0	37	18	348	213	102	103
NP + C3 (PA)	4,67	4,65	10,6	11,4	4,1	2,1	39	20	316	230	138	152
LSD_{05}	0,15	0,14	0,86	$F_ф<F_т$	0,79	$F_ф<F_т$	5	$F_ф<F_т$	85	51	33	12

Table 5 presents the data on fertilizers application effect on the agrochemical parameters of the soil. In both years of research, the value of soil acidity (pH) did not change significantly and averaged 4.7. There was one exception in the treatment with autumn cinder application, where in 2020 the pH value was 4.83, which is significantly higher than the pH value in the control – 4.63. The cinder contains compounds of Ca and Mg, which, when applied in autumn, can pass to the spring in forms accessible to plants and be assimilated during the subsequent growing season. This, however, is not confirmed by the data on the content of Ca and Mg in the soil, which did not differ significantly from the control variant. In studies related to soil fertility indicators, it is difficult to make any definite conclusions based on two years of research, longer observations are required.

The content of mineral nitrogen in 2019 did not change significantly under the influence of the basic application of full mineral fertilizer (NPK), but increased on the NP background and in treatments with spring pre-planting application, which was quite expected. In 2020 the difference between treatments was within experimental error ($F_ф<F_т$). Nitrogen compounds are characterized by increased solubility in soil and are quickly absorbed by plants, therefore, the aftereffect of N fertilizers for the next year was not observed.

The P_2O_5 content slightly increased in 2019 on the NP background and reached the maximum value in the experiment - 432 mg per kg^{-1} of soil, which is higher compared with the control by 70 mg, but within the experimental error. In the other treatments, the difference was even less significant. The same picture was observed in 2020.

The content of K_2O in treatments with traditional mineral fertilizers did not differ significantly from the control variant. The use of cinder, both in spring and autumn, led to a noticeable increase in K_2O in the soil. The raising of K_2O content compared with the control after autumn application was equal to LSD_{05} value (33 mg) in 2019 and reached 183 mg in 2020 (maximum in the experiment). In the treatment with spring application the difference was insignificant (22 mg) in 2019, but next year was reached 37 mg (LSD_{05} value 12 mg). The total K_2O content was higher in 2020 compared with 2019 for both these treatments. This trend can be explained by the slow release of potassium from poorly soluble compounds in the cinder composition.

CONCLUSIONS

Preliminary studies on potato have confirmed the positioning of the cinder as a complex slow-release fertilizer, containing in its composition trace elements Cu, Zn and others, as well as minerals - ameliorants (Ca and Mg compounds).

The direct effect of unconventional mineral fertilizer (cinder) with its pre-planting application was not inferior to traditional potassium chloride fertilization (yield in 2019 – 46.0 and 47.1 t^{-1} ha, respectively).The use of cinder did not have a significant effect on the quality indicators of potato tubers both directly in the year of fertilization and after the storage period. Cinder, in contrast to the standard KCl fertilizer, had a positive effect on the content of exchangeable potassium in the soil. Research with unconventional mineral fertilizers based on K-Mg ore processing wastes should be continued with an emphasis on the study of long-term action as a complex ameliorant.

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THE ADSORPTION CHARACTER OF PERLITE, INFLUENCE ON NITROGEN DYNAMICS IN SOIL

SUMMARY

In this research the sorption of the cations NH_4^+, Al^{3+}, Fe^{2+}, Mn^{2+}, and Mg^{2+} in powdered perlite and its mixture with soil was monitored. Of all observed cations in perlite by the first minutes of laboratory experiments, which is very fast, the ammonia sorption cations reacted fastest. The dynamics of nitrate and ammonia nitrogen in the soil after application of perlite in different quantities were observed. Reduced ammonia content was apparent in variants with perlite one (1) month of application. Three (3) months later, distributed ammonium content increased in variants with perlite from 24% to 59%, compared with the control variant due to the gradual release of ammonium perlite lattice. Six (6) months after the application, statistically significant differences were found not only between the control variant and variants with perlite, but also between the individual variants with different perlite doses ($n = 13.2$; $P = 0.003$ according to Kruskal-Wallis test). The process of nitrification in the soil was strong because of the applied perlite. The content of nitrate nitrogen in the soil is reduced by 66% to 78% compared to control variation in the fall time; therefore, the amount of nitrate leaching from soil horizons groundwater is minimal. In summary, perlite can be considered to be a slow release nitrogen fertilizer. This research concluded that the perlite can find application in the production of mineral fertilizers that is gradually giving the necessary cations.

Key words: Ammonium, nitrate, soil nitrogen dynamics, perlite.

INTRODUCTION

Perlite is a 100% natural siliceous volcanic glass mineral, which traps crystalline water into its mass. Perlite expands when rapidly heated in temperatures of 700°C–1100°C (Dogan and Alkan, 2004). The heating causes entrapped water molecules in the rock to turn to steam and expand the particles.

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Controlled rise of temperature forms a white mass of minuscule glass bubbles. Perlite melts and expands in an extremely porous surface and increasing its volume up to 4-20 times of its original volume (Ennis, 2011). It is very porous, has a strong capillary action and can hold 3–4 times its weight in water. (Bures *et al*. 1997a). This microstructure gives the material a set of favourable properties such as excellent insulation properties, low density and high porosity (Sengul *et al*., 2011; Kramar and Bindiganavile 2013; Polat *et al*., 2015; Markoska *et al*., 2018). We recorded a number of advantages of perlite over other substrates like stability, properties such as: ultra-lightweight, excellent water retention up to four times its weight, advanced drainage and aeration, pH natural and asbestos free, chemically inert, sterile, free of weeds and permanent, serves as an insulator to reduce extreme soil temperature fluctuations, reduces concentrations of salt and also promotes the long term effect of fertilizers (Raviv, M., and Lieth, J, H, 2008; Asher B.,T *et al*., 2008). Moreover, it is commonly used in the food industry, filter product, growing of seed, regulating of the soil in agriculture, and in so many other industrial applications (Alihosseini *et al*., 2010).

Perlite has very good physical characteristics. The physical properties of container-growing substrates, particularly air space, container capacity, and bulk density, have a significant impact on plant growth, and knowledge of these properties is essential in properly managing nursery irrigation and fertilization programs (Yeager *et al*., 2000; Markoska *et al*., 2018). Because of their extremely high sorptive capacity, which depends on the dominant mineral, perlite can be used in different fields, which include agriculture.

Perlite is used in agriculture as a fungicide, herbicide, or plant nutrient carrier. This is feasible simply because of the high sorptive capacity of this rock. When nutrients are introduced into the soil in this way, their consumption rate is reduced, so there is no further need for redundant delivery of raw materials and consequently fewer nutrients (mostly nitrogen, which causes eutrophication of water sources) are leached into ground and surface waters Markoska, 2019; Markoska *et al*., 2018). The atmospheric complex can be divided into two groups: basic cations or more simply bases (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) and acid cations H^{+} and Al^{3+}). Different ions have different energy of adsorption, and they are attracted from the colloidal system by different forces.

Perlite has a high content of total or total adsorption capacity with an average value of 173.32 cmol (+)kg^{-1}. The influence of capacity in perlite is probably due to the mineral composition, resulting from the surface reactions that occur during treatment with acid solution, the part that participates in substitution adsorption. (Markoska, 2019).

The authors Dogan and Alkan (2004) in their study proved that during the thermal treatments of perlite there is a structural transition from amorphous to crystalline, accompanied by increased cation exchange capacity from 20-30 to 35-50 cmol (+) kg^{-1}, as a result of increased specific surface area from 1.2 to 2.3 m^2/g.

Figure 1. Powdered and Raw Perlite (Photo: Markoska, 2020)

MATERIAL AND METHODS

The experimental part served to determine the influence of perlite on nitrogen adsorption and its dynamics in soil. The experimental part was divided into two parts: field part and laboratory part. The used perlite originates from Cerava Poliana, Mariovo Gradesnica, Republic of North Macedonia, and was applied in expanded (commercial) form.

The analysis of the chemical composition of the examined mineral raw material is shown in (Tables 1 and 2). A similar chemical composition is determined in researches of many authors: Herskovitch *et al*. (1995); Uemura *et al*. (1999); Jing, Fang, Liu, & Liu (2011). In the Table 1 we presented chemical composition of perlite.

Table 1. Chemical composition of perlite

Oxides	**Mass percent [%]**
SiO_2	75.47
Al_2O_3	12.77
Fe_2O_3	0.94
CaO	0.61
MgO	0.10
Na_2O	3.04
K_2O	4.51
TiO_2	0.20
Loss of mass	2.36
Total	100

Based on the results obtained from the examinations of the chemical composition of the perlite raw material, it can be concluded that the content of SiO_2 in relation to the other oxides is dominant and is 75.47%. The loss of combustion is the adsorbed water in the material, which is 2.36%. Raw perlite has a low percentage of Fe_2O_3 (0.94%).

The presence of non-ferrous metal oxides is very small. From the results obtained by analysis of trace elements in the raw material perlite, it can be concluded that it also contains low concentrations of As, Cd, Cr, Pb, Mn, Ni, Cu, Cr, B. The trace element can be defined as an element that is represented in the rock in concentrations less than 0.1wt % which is less than 1,000 ppm. Determining the chemical composition is essential because it provides an opportunity to assess the source of the elements present in the raw material itself.

Instrumented Chemical Analyses - ICPA apertures instrument is used to determine the chemical composition of raw perlite. As a consequence of the results of laboratory experiments, the effect of perlite on the dynamics of mineral nitrogen in soil with a depth of 0.0–0.3 m was verified in field experiments.

Selected chemical soil properties were determined using the obligatory methods for soil analysis (Mitrikeski *et al*., 2013).

There were 4 variants of the experiment:

Q, control variant without any fertilization;
Perlite 1, 500 kg of perlite type per hectare;
Perlite 2, 800 kg of perlite type per hectare and
Perlite 3, 1000 kg of perlite per hectare.

All variants were carried out 4 times. The area of the experimental field was 50 m^2 (10 x 5m). The experimental crop was medium-ripening white tomato (*Solanum melogena*) grown with the usual agricultural techniques. Seedlings were planted on 15 April and harvested on 31 August 2019. Average soil samples were taken and analysed for pH, nitrogen (ammonia and nitrate), and the contents of available phosphorus, potassium, magnesium, before the experiment was set up and after its completion on each site. In addition, once a month, soil samples from a depth of 0.0–0.3 m were taken from each site in order to monitor the dynamics of ammonia and nitrate nitrogen in the soil. The statistical processing assumed that the perlite dose influenced both ammonium and nitrate content in the soil. The Kruskal–Wallis test was chosen for verification of this hypothesis. In the case of rejection of the null hypothesis, the non-parametric Mann–Whitney test was used.

RESULTS AND DISCUSSION

The exchangeable perlite cations Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} are already present in natural deposits. These cations can be exchanged by NH_4^{+} and other metal cations, depending on the pH value of the solution and their concentrations.

The time-dependent development of NH_4^{+} sorption from solution in perlite is shown in Figure 2, where the curve reflects the time dependence of the sorption of the initial NH_4^{+} concentration.

The development of the sorption indicates a very rapid response and, in the first minute, a sorption of more than 90% of the maximum potential ammonia concentration in perlite on an infinite timescale can be observed. During the next

period from 2 min up to several hours, the concentration of NH_4^+ does not change significantly and is almost constant.

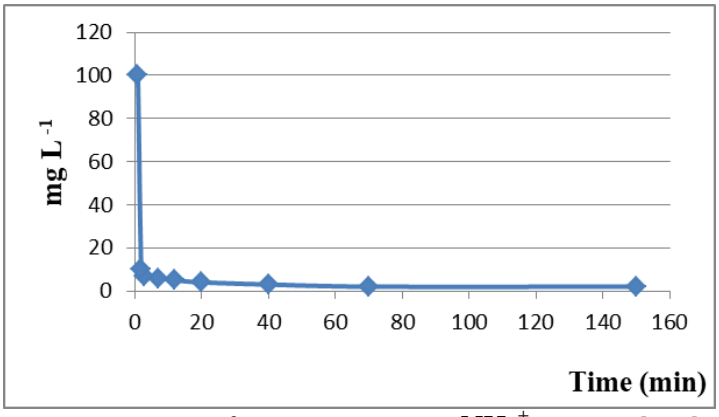

Figure 2. Sorption rate of ammonia cations (NH_4^+) on powdered perlite (0.1 kg L^{-1}, fraction under 45 pm).

Figure 3 presents the rate of sorption of the other observed cations. Similar to the sorption rate of the ammonia cations, the iron cation sorption (Fe^{2+}) was very fast.

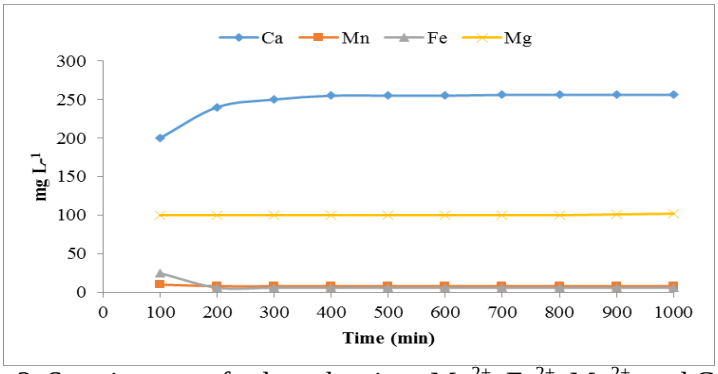

Figure 3. Sorption rate of selected cations Mg^{2+}, Fe^{2+}, Mn^{2+}, and Ca^{2+} on powdered perlite (0.1 kg L^{-1}, fraction 45 μm)

A significant decrease in Fe^{2+} concentration could also be caused by Fe^{2+} oxidation to Fe^{3+}, and it is coagulated at filtration. Manganese (Mn^{2+}) sorption was also fast in the first minute, but as manganese has less sorption selectivity than perlite, no significant reduction in concentration occurs, only approximately 40%. The calcium (Ca^{2+}) concentration in solution did not have a tendency to increase by time. The concentration of Mg^{2+} was initially slightly decreased and then increased slightly later. On the basis of experiments in which the time dependence of NH_4^+, Fe^{2+}, Mn^{2+}, Ca^{2+}, and Mg^{2+} sorption in the perlite powder

was measured, it can be concluded that the sorption of the cations is extraordinarily fast.

This is illustrated by the fact that in the first minute sorption in perlite reached more than 90% of the maximum possible concentration of equilibrium, which would stabilise in infinite time. It can be assumed, however, that this processes depend on the sizes of the perlite particles, so when they increase, the stabilisation of sorption equilibrium, and especially that of NH_4^+, will be slower. The stabilisation of ionic exchange balance in several minutes or hours can be considered as fast because the perlite will be in the soil for several years after its application. This knowledge can be applied to liquid nitrogen-based plant nutrition on the basis that perlite applied to the soil would prevent the escape of redundant ammonium ions, as these would be bound to perlite in moist soil very quickly and would be gradually released into the soil solution.

The observed cation solution was verified by a field experiment based on Vertisols (FAO Classification, 2006); (WRB Classification, 2016) which the basic chemical properties are shown in Table 2 (nutrient analyses according to the Mehlich method, organic carbon according to the Tjurin method).

Table 2. Physics-mechanical and chemical soil parameters and clay content in the soil Vertisol.

Depth in m	pH in KCl	pH in H_2O	N-NO_3 [mg kg^{-1}]	N-NH_4^+ [mg kg^{-1}]	N an	N $_{total}$	P	K	Mg	Silt + Clay < 0.02 [mm]
0-0.3	7.1	7.9	31.3	23.8	55.1	1743	88.0	381.0	136.0	65.50
0.3-0.6	7.1	8.0	21.6	24.8	46.4	1417	59.0	264.0	143.0	60.30

In Figure 4, the dynamics of NH_4^+ in the soil is presented. It can be seen that after 1 month of perlite application to the soil, the Vertisols topsoil contained 14 % to 20 % more ammonia nitrogen (91.5 mg kg^{-1} of soil) in the control variant compared with the experimental variants (76.2 – 80.0 mg kg^{-1} of soil).

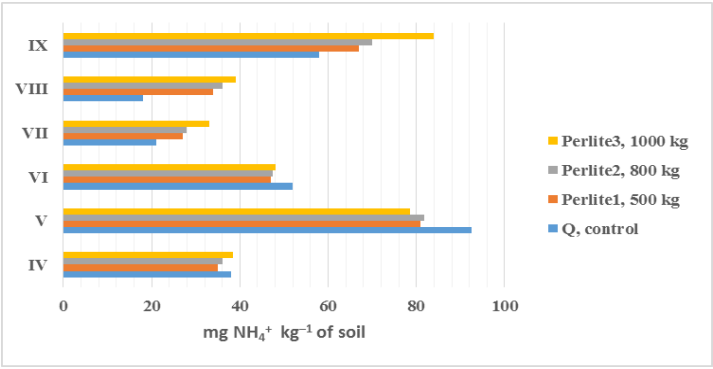

Figure 4. Dynamics of ammonia nitrogen in the soil horizon 0.0–0.3 m after the application of different amounts of perlite

There was a change in this subsequent period; the content of ammonia nitrogen in the topsoil in the variants with applied perlite increased and after 3 months reached 24 % to 59% higher values (depending on the amount of perlite) than the control variant. At the end of the field experiment (5 months after the application of perlite), the experimental variants reached 66.5–84.9 mg, and the control variant reached only 53.2 mg per kilogram of soil.

From the calculated values of the Mann–Whitney test, we can conclude that statistically significant differences in mean NH_4^+ concentrations in the soil were confirmed not only between the control variant and the observed ones, but between all observed variants, as well (Table 3).

Statistical analysis of the results according to the Kruskal–Wallis test confirmed the significant differences between the mean values of all observed variants (H =13.2 with P = 0.003). The Mann–Whitney test results are given in Table 3. After the perlite application, the nitrate nitrogen content also changed during the observed period (Figure 5). All variants kept the spring maximum in April (23.3–26.5 mg kg^{-1} of soil) and the summer minimum in June and July (1.3–4.8 mg kg^{-1} of soil). At the time of crop harvest (in August) there was an apparent increase in nitrate nitrogen (10 mg kg^{-1} of soil) in the control variant, which corresponded to the so-called autumn maximum. This was not confirmed in the variants with perlite because the nitrification process of ammonia nitrogen, owing to the presence of perlite in the soil, becomes slower.

Table 3. The main statistical parameters from the last measurement at the end of the field experiment (ammonia nitrogen).

	Control variant	500 kg perlite ha^{-1}	800 kg perlite ha^{-1}	1000 kg perlite ha^{-1}
Minimum	52.50	65.30	67.40	84.10
Maximum	54.10	66.90	69.50	85.40
Median	53.20	66.90	69.25	85.05
Arithmetic mean	53.25	66.50	69.10	84.90
Standard deviation	0.633	0.700	0.383	0.472

Table 4. Mann–Whitney test results of the observed variants (regarding ammonium nitrogen).

	500 kg perlite ha^{-1}	800 kg perlite ha^{-1}	1000 kg perlite ha^{-1}
Control variant	0.000^+ (0.03)	0.000^+(0.03)	0.000^+ (0.03)
600 kg perlite ha^{-1}		0.000^+(0.03)	0.000^+ (0.03)
900 kg perlite ha^{-1}			0.000^+ (0.03)

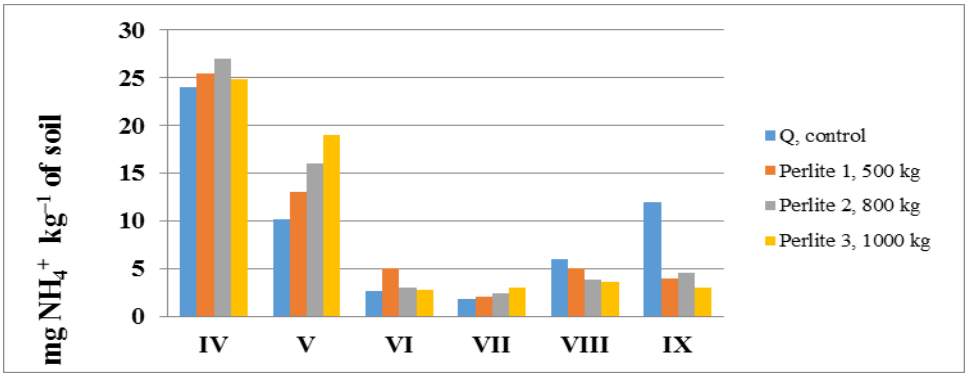

Figure 5. Dynamics of nitrate nitrogen in the soil horizon 0.0–0.3 m after the application of different amounts of perlite

From this perspective, the application of perlite holds back the chemical change of ammonium cations into nitrate anions, thereby decreasing the amount of nitrate leaching into the groundwater. In this way, it is possible to significantly reduce nitrogen loss, which reaches, according to several authors, 25%–35% of the total amount of applied nitrogen in mineral fertilizers.

In our case, the content of nitrate nitrogen in the soil in the experimental variants was 66% to 78% lower in comparison with the control variant. Statistical analysis of the results according to the Kruskal–Wallis test confirmedthe significant differences between the mean values of all observed variants (H =12.7 with P =0.003). The Mann–Whitney test results are given in Table 5.

Table 5. Mann–Whitney test results of the observed variants (regarding nitrate nitrogen).

	500 kg perlite ha^{-1}	800 kg perlite ha^{-1}	1000 kg perlite ha^{-1}
Control variant	0.000^+ (0.03)	0.000 (0.03)	0.000^+ (0.03)
500 kg perlite ha^{-1}		1.500^- (0.6)	0.000^+ (0.03)
800 kg perlite ha^{-1}			0.000^+ (0.03)

Stabilization of the exchange equilibrium was reached in only a few hours. Our results showed a reduction of ammonia nitrogen content in the soil a short time after perlite application. This reduction of the contents in the experimental variants can probably be attributed to fixation of ammonium ions by specific areas in the perlite crystal lattice, because they bind to the perlite with the greatest intensity of all cations (Markoska, 2019).

During the 5 months of the experiment, we found higher contents of ammonia nitrogen in the variants with perlite, which means ammonia nitrogen is gradually released from perlite and more is available for cultivated plants. Application of 2 t of perlite in heavy soils increased the hydrolysable nitrogen

content twice, and when they used 4 and 6 t of perlite, it increased by 4 and 5 times, respectively.

The fact that perlite can relatively strongly fix the ammonia nitrogen leads to the reduction of nitrogen leaching. This is confirmed not only by our results, but also by researchers in many countries who have had positive results with perlite application decreasing nitrogen loss due to leaching (Işıldar, 1999; Gholamhoseini *et al*., 2009; Ippolito *et al*., 2011). Perlite is also used to remove nitrates from water (Mazeikiene *et al*. 2010). In their experiments there was an observed decrease of the $N\text{-}NO_3^-$ in the soil only to the level of 41%–48% after the perlite application compared with the nitrate nitrogen content in the control variant. Whereas at the beginning of the experiment statistically significant differences were confirmed only between the control variant and observed variants, in the last month of the experiment they were also confirmed between all observed variants. This was confirmed by a Kruskal Wallistest with a relatively high level of significance ($H = 12.7$ and $P = 0.003$). Regarding the obtained results, it is possible to conclude that sorption of cations NH_4^+, Fe^{2+}, Mn^{2+}, Ca^{2+}, and Mg^{2+} in perlite with a grain size of less than 45 μm takes place very quickly. More than 90% of the maximum possible concentration of those cations is sorbed in the first few minutes.

CONCLUSIONS

The dynamics of ammonia nitrogen in the soil during the 5 months of evaluation endorse perlite as slow-releasing nitrogen fertiliser. It has a significant effect in the prevention of nitrogen loss from soil. Short time after perlite application, the nitrogen content in the evaluated soil decreased as a result of NH_4^+ fixation by perlite, the increase was probably caused by the gradual release of nitrogen from the perlite crystal lattice. Therefore, the perlite works in the soil as a sort of slow-releasing nitrogen fertilizer with NH_4^+ ions and indirectly limits the intensity of nitrification in the soil. Thus provided a sufficient amount of nitrogen throughout the vegetation period for cultivated plants. On the other hand, the presence of perlite in the soil in habits the process of nitrification to a certain extent, so that the nitrate leaching into deeper soil horizons is not so intensive. Perlite application to soil favorably affects the environment by preventing the leakage of mineral nutrients (especially nitrogen) in to groundwater and increasing the efficiency of nutrients occurring in soil, and it does not negatively affect the quality of the cultivated products. It is fair to say that natural perlite plays a positive role in plant nutrition.

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ANTI-EVOLUTION OF THE PHENOMENON OF CONSTRUCTION-ITS RELATION TO THE ENVIRONMENT

SUMMARY

The phenomenon of construction, as an act of creation of a man-builder in the landscapes, is being discussed in this paper. Vernacular architecture is the creation of the epochal relation of man to the environment. The coexistence of man and nature has been transformed into architecture that still goes beyond the reach of contemporary construction. Evolutionary processes have been called into question, which justifies the concern of the international community regarding the protection of the so-called cultural landscapes, places of conflict between nature and man. What is the perspective of these creations and are the builders of today familiar with the postulates and laws of creating the dwelling architecture of their predecessors? The paper elaborates general principles of protection and emphasizes the importance of preserving the rarity of the traditional opus of construction related to the local examples that exist on the territory of Montenegro, which are the works of an unknown builder and which are currently under-researched and under-valorised. The current knowledge base on "ambient nuclei" (Perovic, 2005) presented through the cases of villages in Montenegro does not grasp the complete essence of the architectural thought. Evolutionary processes of the permeation of natural processes with modus of construction and the current stagnation of processes demonstrated on the examples of villages Godinje and Gornja Brca, located on slopes not far from the water, have been considered in this paper. Man has affected the nature by the phenomenon of construction, creating the unique cultural landscapes. Man has used nature as a resource. The evolution of man in the natural environment implied the evolution of nature itself, natural processes flowed simultaneously. Balance is achieved through synergy. However, there was a point in time when nature continued its life without man, because natural forces have overpowered the human creation.

Keywords: evolution; creation; cultural landscape; dwelling; natural processes.

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1. Introduction

Vernacular architecture as an act of creation by unknown man

The topic of the paper is the reach of vernacular architecture, which is a reflection of man's aspiration to create conditions for existence in nature and to improve the conditions of his life by continually unconscious architecture. It is an unpretentious, "modest" architecture, without the urge to dominate the natural environment, based on human intuition. An architecture that adapts to the man and the natural environment, as a contrast to today's architecture, which tends to dominate nature.

The paper will focus on the achievements of *vernacular architecture in the territory of Montenegro.* The study of the phenomenon of vernacular architecture is based on the examples of the Montenegrin villages of Godinje and Gornja Brca. The thread connecting these two places is the uniqueness of the cultural landscape and the response of the unknown man-builder to the established natural context. The natural context of *Godinje* and *Gornja Brca* is similar; both are positioned on a slope and close to the water, seen as a source of life. The builder's response is also unique; both "ambient nuclei" have the views of the lake or the sea, but at the same time they are distanced from the water for easier defence against the enemy. The aim of the research is to define certain principles and postulates in the process of construction – the creation of vernacular architecture. *Can postulates of vernacular architecture be implemented by modern architects when creating?*

Figure 1. Cultural landscape, village Gornja Brca

The etymology of the concept of vernacular architecture is interpreted by Darko Radovic and is linked to Roman law in which the term vernacular denotes the opposite of the goods being purchased. Vernacular is a Latin word and its basic sense refers to "A slave born in my house by a slave whom I own" (Radovic, 2005). Radovic also quotes the Roman grammarian Vara, who, by the term vernacular means indigenous words.

In his book *Architecture without architects*, Rudofsky Bernard defines vernacular architecture as a timeless creation that does not pass through the fashion cycles and which, above all, satisfies the basic human need for space, adding that: "*As a rule, the origins of indigenous building forms and constructive methods is lost and represents a part distant past. Buried below typical Mediterranean houses*". (Rudofsky, 1985).

Vernacular architecture is a product of spontaneity and intuition; it is not the answer of a thoughtful architectural approach of an architect, driven by the knowledge of the theory of architecture, urbanism, and psychology of perception, with an understanding of the law of the unity. The question then arises as to how the ambient entities were then interpolated into landscapes. *Who is their builder?*

The answer may also be found in Le Corbusier's "*story from the east*" about the art of the villagers, who create traditional art embodied in a vase form. Describing vases, Le Corbusier speaks of a person's innate aesthetic sense to connect line, colour, abstract performances, aesthetic values, and unconsciously create a work of art, relying on the inner intuition and creative force that exists in each person, as well as the innate sense of harmony, because in the end, man himself is a product of natural harmony, "*The peasants' art is an astonishing creation of aesthetic sensuality*." (Le Corbusier, 2008)

If we would talk about successful architecture of peasants, we would find a certain principle in the method of multiplication, a certain repetition of the adopted model of a house for a certain environment, whereby the thinking man-builder would notice the defects of a certain model and during the next construction he would eliminate the deficiencies and refine the model until he unconsciously reaches the level of architectural realization, striving exclusively to creating a safe existential space. On the other hand, out of the need for less expenditure, he thoughtfully used the natural context and ambience. Dom Hans van der Laan will, regarding the aforementioned house-context relation, say that the house is the place of our encounter with nature, that it is actually a "*slice of living space that we extract from the natural environment by constructing the walls*". (Dom Hans van der Laan, 2009)

However, today's relation between the man and the nature is characterized by the endeavour of man to master the nature, to subjugate it, to show, by the work which he creates, superiority over it. *The paradigm of today is "Architecture - the ultimate victory over nature."* Even in cases where the work follows the postulates of organic architecture, it still reflects man's triumph over nature and its inferiority, when the builder exploits and uncritically consumes natural resources; he usurps sites with attractive natural and cultural characteristics.

2.The act of building - about the nature of things
Who builds houses, is it just a "homo- aedificator?"

Figure 2. Anthill- ants construction*

Figure 3. Godinje - man's construction

** From the book of Pađan, Z. (2005): Arhitektura prirode, Zagreb, Školska knjiga*

In rural areas, the land has ultimate ecological, social and cultural value (Kosanovic *et al*, 2016). In agrarian landscape, the need to study nature and culture together is evident (Palang *et al.*, 2006). The patterns humans impose on the earth, purposefully as well as inadvertently, through land-use change are fundamental determinants of local, regional, and global ecological processes that ultimately influence the sustainability of both biological and cultural landscapes, and thus human quality of life. (Redman, 2008; Fikfak. *et al.*, 2017; Parsipour *et al.*, 2019).

In the book by Zvonko Pađan, *Architecture of Nature*, the problem of the act of creating living spaces from prokaryotes to chimpanzees, that is, from organisms without a functional nucleus to organisms that are at the very top of evolutionary development, is meticulously discussed. On that occasion, Pađan will conclude that there is a construction that develops independently of the human construction. With this in mind, the act of construction was not invented by man; construction also exists outside the human community. The essence of construction is the motive of construction, being in harmony with the environment and creating an inner existential space. However, in his exhaustive research, the Croatian architect and scientist did not conclude it with a full stop and he left space for further research on the evolutionary process of construction.

The motive behind the construction is to provide the aspect of safety and comfort elements. Being intermittent with the environment means at the same time enclosing it to create a safe interior space, in which case the construction is based on the principles of biomimicry. Biomimicry involves incorporation of form, materialization and colour into the existing landscape.

Man's construction oeuvre stands out because he possesses an artistic line and does not merely satisfy the needs of existence. Man builds from his cultural and spiritual initiatives and thus stands out as the higher life form on Earth. In his research, Pađan will distinguish the construction of man and lower beings by saying: "While in the lower beings a narrow, genetic, hermetic program prevails, in a man's case, it is an open act of creation." (Pađan, 2005)

However, there is an order of builders in the human community as well. Vitruvius described the architect, the first builder, focusing on his education with the words: "*He must still be adept at pen and drawing, he must have a good understanding of geometry, and know history well, to listen to philosophers diligently, to know music, to not be ignorant in medicine either, understands legal issues, and knows something about astronomy and the laws of heaven.*" Only after studying these scientific areas did the builder reach the "holy ground of architecture". (Vitruvius, 2009)

Examples of human construction, which are discussed further in the paper, are exceptions where Vitruvius' view on the education of an architect is not valid. *We shall observe the act of construction as a genetic program that develops inside of a man, and what sets man's construction process apart from other builders is the innate aesthetic thought and awareness of the possible controlled use of the natural resources of the environment.*

3. From general to regional- International systems for protection and valorisation of cultural landscapes

A series of photographs by Bernard Rudofky at the Museum of Modern Art in New York had marked the year of 1964. It was the autumn when *Architecture without an Architect* intrigued the expert public and provoked numerous polemics on the relationship of today's builders to the environment. An overview of the international scene and the existence of vernacular structures outside the territory of Montenegro will emphasize common principles of construction in the natural environment and the primacy of strategic protection of them. The cases of villages of Godinje and Brca will find an analogy with Italian towns settled on the slopes – Italian hill town, with the vernacular architecture of Apanomeria, a remarkable example where nature stimulated human creative power - classical vernacular and with the precursor of bioclimatic urbanism, the covered streets of cities in Spain and the Libyan Desert - covered streets.

By analyzing the above examples, we can conclude that the reach of vernacular architecture is timeless and extraterritorial, that the principles, ideas and thought underlying the vernacular architecture are general, and refined and materialized depending on the context.

Considering the architectural heritage of vernacular architecture and landscapes as significant, numerous international conventions address the issues of their protection and valorisation, emphasizing the importance of integral protection and increasingly emphasizing the importance of cultural landscapes.

The recommendation on the protection of historical and traditional entities and their role in contemporary life defines the concepts of environment and protection. "The environment" of historical or traditional entities means a natural or constructed framework that influences the static and dynamic perception of these entities or is directly related to them in space, that is, natural, social or cultural connections. "Protection" means the identification, protection, conservation, restoration, rehabilitation, maintenance and revitalization of historical and traditional entities and their surroundings." (Nairobi, November 1976).

The Convention for the Protection of the Architectural Heritage of Europe defines "localities as common works of a man and nature, or areas partly built and sufficiently distinctive and homogeneous" as the architectural heritage (Granada, October 1985).

European Convention on the Landscape of the Council of Europe "Landscape is defined as a specific territory, as viewed by the population, whose character is the result of the actions and interactions of natural and / or anthropogenic factors." (Florence, October 2000). According to the definition formulated by UNESCO, "Landscape cultures are a combination of the effects of nature and man and illustrate the development of human society and its settlements over time under the influence of physical forces and / or the opportunities that represented their both external and an internal environment affected by social, economic and cultural forces. "

The Council of Europe Framework Convention on the value of cultural heritage for society introduces the concept of a common European heritage, consisting of "all forms of cultural heritage in Europe which collectively form a common source of memory, understanding, identity, cohesion as well as ideals, principles, values derived from the lessons learned during the progress and conflicts in the past." (Faro, October 2005).

Since the architectural heritage of the traditional relationship between architecture and landscapes in the Mediterranean area is very characteristic, the *Euro-Mediterranean Charter was adopted in Rome in 2002* on the integrative valorisation of cultural heritage, relying on the historical and geographical link between the Mediterranean heritage and emphasizing the importance of establishing a new cultural heritage management strategy based on integrative planning.

Indigenous building forms and constructive methods therefore were not lost under the Mediterranean houses. Their values are recognized, internationally recognized, with the intention of upgrading Euro-Mediterranean identity with the goal of planned tourism development through comprehensive planning and valorisation. The Mediterranean House is still recognized today as a par excellence example of construction in the natural environment of the Mediterranean, where sea, stone and vegetation stimulate creativity during the act of construction.

4. From regional to local - National systems for protection and valorisation of cultural landscapes

There is significant number of examples of vernacular architecture in the territory of Montenegro in areas with outstanding natural values. *The synergy of a man-builder and nature has produced in the territory of only 13,812 km² a variety of cultural landscapes on a scale from Mediterranean to mountain, Dinaric Alps in the north of Montenegro.*

*The Spatial Plan of Montenegro until 2020***,** which was adopted in 2008, recognizes 21 landscape unit, and the Skadar Lake area is one of them, where Godinje is also located (Ljeskovic, Mitrovic, 2012). While Gornja Brca is located between Bar and Sutomore, not far from the sea coast. Montenegro also ratified the European Landscape Convention in 2008, when it set out to implement the convention at a national level, recognizing the importance of protecting, managing and planning landscapes at national and interstate levels. (Kujundzic and Stamatovic Vuckovic, 2019)

The laws and strategies governing the protection of cultural landscapes at the national level are: *Law on Spatial Planning and Construction of Facilities* ("Official Gazette of Montenegro", No. 064/17 of 06.10.2017, 044/18 of 06.07.2018, 063/18 of 28.09 .2018); *Law on the Marine Property* ("Official Gazette of the Republic of Montenegro", Nos. 14/92, 59/92 and 27/94, Official Gazette of Montenegro ", Nos. 51/08, 21/09, 73/10 and 40/11*); Law on Nature Conservation* ("Official Gazette of Montenegro", No. 054/16 of 15.08.2016); *Law on Protection of Cultural Property* ("Official Gazette of Montenegro", No. 049/10 of 13.08.2010, 040 / 11 of 08.08.2011, 044/17 of 06.07.2017, 018/19 of 22.03.2019); *National Sustainable Development Strategy until 2030* and a number of other laws and by-laws that tangent to the field of protection of cultural landscapes. Protection system is, in theory, based on the networking of complementary laws.

We can see Montenegro's tendency to establish effective, adapted and focused legislation on the issues of integral protection and connection of architectural heritage with spatial planning, which will avoid secular access to cultural heritage and utter commercialization of cultural heritage, while simultaneously giving life to cultural and natural resources. *The protection of the cultural landscape and its integration for tourism purposes is a challenge for both citizens and competent national authorities.*

The work program of the Government of Montenegro for 2011 envisaged and implemented the reform of cultural institutions dealing with the protection of cultural property into a new, as stated by the legal act - oriented, institutional framework, *which is based on the sustainable use of cultural heritage***.** In order to successfully valorise cultural heritage, the need for a thoughtful revitalization of the architectural heritage space is increasingly emphasized, giving it a purpose that suits the needs of modern man. Only spaces and environments with recognized values can become the framework for contemporary lifestyles and the context of contemporary construction. In this way, the Ministry of Culture with

the protection services, also contributes to the establishment of national guidelines for the development of architecture, giving context to contemporary architecture.

Finding a universal solution to the problem of revitalizing the architectural heritage of vernacular architecture and *deus ex machina* is not possible. Each cultural landscape carries with it certain social, cultural and natural peculiarities and complexities, which first must be noticed and analysed.

5.1. Village Godinje – the coexistence of man and nature

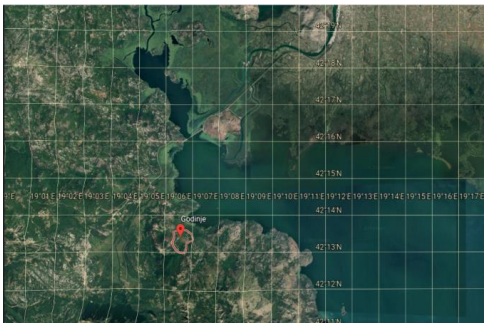

Figure 4. Location of the Godinje

Figure 5. A view of Skadar Lake

"Then move on to another house - two children have appeared. Then you need to accommodate four children. As all this is very difficult, first draw a straight line around which you will build all the necessary units in the proper order, each on the smallest possible surface. Then, as in the form of a genealogy, figure out where they shall be, by arranging the corresponding units one after the other."(Le Corbusier, 2009).

The ambient unit of Godinje acquired the status of cultural property in early 2016. The image of a medieval town from the late XIV century, near Lake Skadar, is now made up of walls, traces of matrices and once dominant structures, partly preserved in their authentic form.

Figure 6. Roofs in village Godinje

Figure 7. Architectural remains of village Godinje

The identity of the place is contained in the unique colour, landscape and architectural style elements, which represent inherited values and are recognized as such, with a tendency to be nurtured and protected. Group forms of ambient nucleus houses remind us of a previous way of life and represent a kind of open-air museums, in which the main exhibits are works of vernacular architecture, by the unknown man-builders. Several households still live in Godinje today, while the largest migration occurred after the devastating 1979 earthquake.

Godinje is located on a limestone hill not far from Skadar Lake with a clear view of the lake itself, while the village is impossible to be seen from the lake. It represents a settlement of the Lekovic fraternity, where the houses are positioned in the form of genealogies, stacked on top of one another. Natural and anthropogenic factors competed in this cultural landscape. The houses functionally and content-wise represent a combination of place of residence and economic space for storing food, making wine, conditioned by the configuration of the terrain and leaning on each other. The vine is the light motif of Godinje.

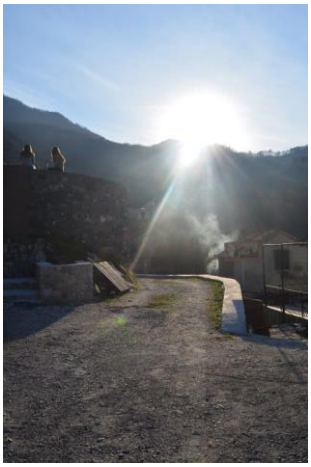

Figure 8. Gumno

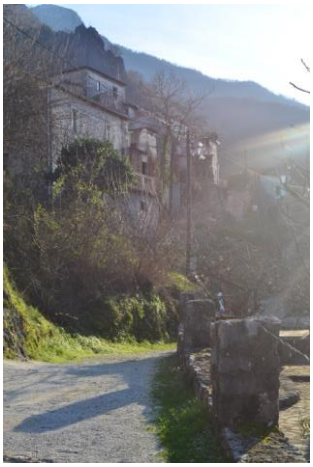

Figure 9. Dwellings

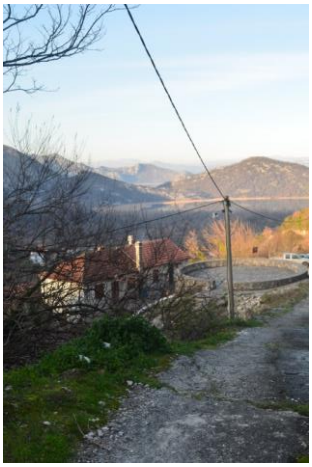

Figure 10. Center

To the Godinje ambient nucleus can be given the epitome of urban, primarily because of the present collectivism, the tightness of houses, clear paths, and the gathering place in the form of two outdoor threshing floors, which can replace the city squares. The larger threshing floor, the main square, has a symbolic significance for the locals in the hamlet; it is an intersection - three points, the intersection of three paths, which are parallel to the isohypses. All three come out to the stream, which houses the mills and wheels from which the hamlet was supplied with water.

If we were to analyze the house of Godinje as the basic unit of the settlement-archetype, in our perceptual field we would first find a volt, a stone-vaulted semi-open space, as the most characteristic element of a house of Godinje. At the volt there are terraces, intimate open spaces over which one enters the house. The stone houses are covered with gable roof covered with

hollow tiles. There are still under-researched assumptions about secret underground passageways linking houses.

The architecture, which dwelt as a genealogy following the isohypse, interpolated into the distinctive landscape of the limestone massif, using stone as the main form of materialization and natural rock as its support. The nucleus' distinctiveness speaks of a building evolution in solving the problem of construction in the sloping terrain. The inner courtyards are designed meaningfully and represent valuable sub-environments. On the facades of houses, the boundaries of the natural and anthropogenic are incomprehensible.

Figure 11. View from the “square”

Figure 12. Community

Architect theorist Schulz would say that a man "builds what he sees." (Schultz, 2009) Further explaining that man represents his view of nature by construction. During construction, man follows the lines of nature and paths and has the need to translate the experienced meaning of nature and himself in nature into a building. Schultz talks about man's need to accumulate all the experienced meanings to create his own imago mundi, or microcosm. In this way, artificial places are created in the landscapes, as a concretization of man's view of the natural environment.

In Godinje, the air currents smell of the sea, which is mixed with the smell of lake fish, the dominant sound is the murmur of water, which comes from the local faucet, where the locals traditionally clean the fish, there are also rhythmic noises, which are occasionally heard and the silence, which originally comes from the arched volts, echoes through the narrow stairs and passageways, eventually sinking into the stone. It's always cold out there. It’s a place known for its wine taste.

However, the phenomenon of construction has stagnated at some point. In pervading natural processes, nature has conquered the man. Man did not respond to nature's reaction to construction. He deviated from the principles of construction and utilitarianism became the main feature of his construction.

In the absence of an answer, the phenomenon of construction has become a destructive act, leaving behind the lasting devastation of the environment. The spatial values of the architecture of that time remained meaningless, devoid of function.

Figure 13. Arch, today

Figure 14. The power of nature

Some of the basic features of vernacular architecture have been lost. Architecture that seeks to rest on traditional construction is based on imitation. An essential feature of a "healthy" relationship with the environment is lost beneath the old house of Godinje, which remains as a reminder of the act of creation of unknown men-builder. The process of evolution stopped when man decided to wall up half of the arch.

5.2. Gornja Brca village - coexistence of architecture and nature

Figure 15 Location, Gornja Brca

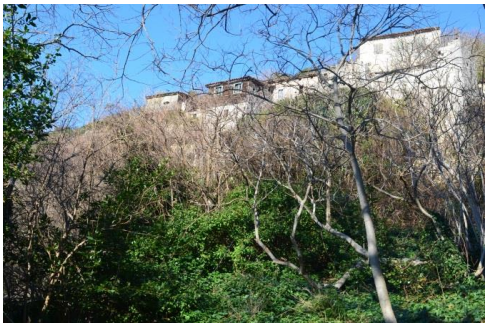

Figure 16. Village Gornja Brca

"The boundaries of the built-up space are known as floor, wall and ceiling. The boundaries of the landscape are structurally similar and consist of earth, horizontal and sky." (Le Corbusier, 2009)

Unlike Godinje, Gornja Brca is not a legally protected environment. In the Study on the Protection of Cultural Property, made for the purpose of developing the Special Purpose Spatial Plan for the coastal area of Montenegro, Gornja Brca was recognized as a good with recorded cultural values.

The village of Gornja Brca is located on a slope in the hinterland, not far from the sea. The background of this village is made of a rocky area. The site was often attacked, so fortification largely conditioned the conception of ambient nucleus development. Searching for a safe habitat, the settlement is located off the fertile foothills and the view to it is impossible from the sea. Today it is an

uninhabited place because the locals have returned to the foothills of the hills, which abound in water and which today is an olive field. Olive is the light motif of Brca.

Figure 17. Olive grove

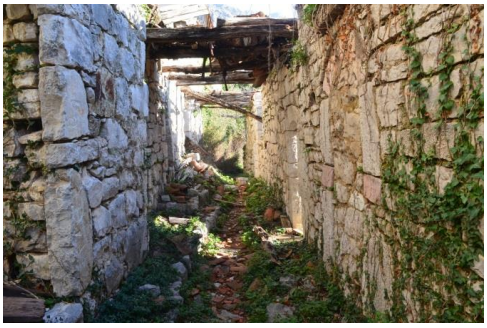

Figure 17. Covered street, Gornja Brca

Urbanism of the ambient unit is conditioned by the configuration of the terrain; the houses are built in a row with common gable walls. A number of houses were created following the development of the family as in Godinje. The houses have southern exposure and follow isohypses, with a pronounced horizontal, which represents nature. A characteristic architectural element is the "covered street", the beginning of bioclimatic urbanism. (Vuksanovic, 1998) The outlines of fortification architecture are observed in the conception of the ambient unit and in the examples of watch-towers with a pronounced vertical, a reflection of human construction.

Figure 19.
Materialization

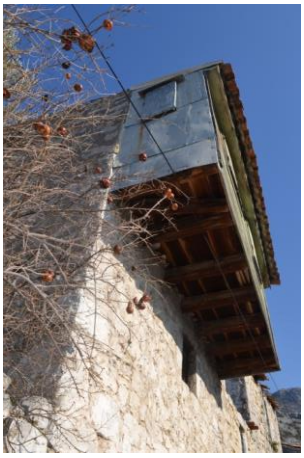

Figure 20.
Console

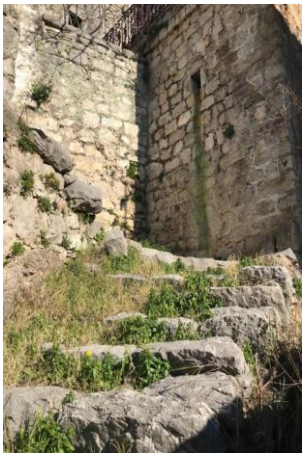

Figure 21.
Pathway in Gornja Brca

The house in Gornja Brca has a single-sloped roof, which follows the slope, like houses in Pastrovici and a stone plinth with a floor frequently materialized with timber. The families who have their roots in this place are

Ilickovic, Luksic and Đuranovic. According to the chronicler, the village belongs to extremely old settlements. (Dudic, 1967)

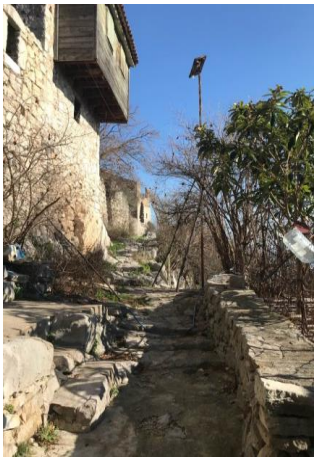

Figure 22.
Detail of single roof

Figure 23.
In between

Figure 24. Detail of console in Gornja Brca

Reminiscence of the old settlement, situated on inaccessible terrain with the intention of being easily defended and inaccessible, now consists of stone walls with tree floors and green details in the form of creepers, which complete the picturesque picture of the settlement, as well as the dominant structures of the tower houses, pronounced cultural layering and spontaneity of construction. Unlike Godinje, Brca is dominated by the smell of the sea, blue colour, karst and wood, which gives the whole picture warmth. It is warm here, noises do not exist, only the voices and sounds of construction machinery from the village at the foot of the hill. As you approach the village, the sound you are recording is a rush of spring water from which the city water supply is supplied. In the memory of the visitor remains a view of the village covered with a shadow of the olive trees. Olives and pomegranates are everywhere; they have also shot up in the covered aisles, closing them. Rock, stone, vegetation, wood spontaneously change on the building. Recent interventions with brick, concrete and subsequently closed openings are also noticeable, again indicating a recent human existence. Now the walls of these houses are lifeless architecture and a mere remembrance of their former existence within the ambient nucleus.

In the example of the village of Gornja Brca, we can see the process of supplementing, upgrading; where man was left unfinished in his creative expression; he was replaced by nature, pomegranate shoot up. Brca is a reminder that stagnation is followed by disappearance; existence is only possible through constant evolutionary processes. The layering of Brca testifies to the man's effort to prolong his existence. After a while, man leaves architecture on a slope, forming existential conditions in a flat, fertile land, as the defence ceases to be a sine qua non.

When the man stops building in space, nature continues its process and masters the man's construction, it settles there.

In her article, Professor Stamatovic Vuckovic defined Gornja Brca as "zero space". By the term "zero space", the professor refers to spaces that bring us back to zero, to the essence of our existence, an essence that is reflected in the synergistic relationship between nature and man. In these spaces one feels a strong connection with nature. The "zero space" returns a man to the beginning and reminds us that it is necessary to reprogram human construction to the primary postulates of construction in harmony with nature. (Stamatovic Vuckovic, 2018)

6. Concluding Remarks

Nullus rogandi finis

Group forms of houses of ambient units, different from the built urban patterns, set to rural areas; created in natural landscapes, have succeeded in developing an honest relationship towards the ambient environment and nature (Fikfak, *et al.*, 2015).

The unknown man-builder was able to create the conditions for existence on an inadequate space, creating authentic modules for shaping space through spontaneity as an allegory of nature.

Figure 25. Abandoned building near village Godinje

It is possible to outline certain principles in the process of construction - the rules of the act of creation in vernacular architecture, which are recognized in both of these examples:

(1) Functionality; (2) Simplicity;
(3) Cost-effectiveness; (4) Bioclimate;
(5) Aesthetics; (6) Humanity

Namely, modesty of all the architectural elements of the house in relation to the nature, following the aforementioned principles, had, as a result, a functional housing architecture based on collectivistic. Man knew, without prior knowledge, that he was responding to the challenges of nature, that is, he was adjusting his process of construction to the processes of evolution. Today is a moment of stagnation, which gravitates toward disappearance. Abandoned and un-valorised works of vernacular architecture exist, but only as ruins occupied by nature, with no man. They are on the earth and exist in landscapes as their integral part and as a reminder of the winning concepts of designing within natural entities.

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ESTIMATES OF SOIL LOSSES IN WATERSHED UNDER TROPICAL OF ALTITUDE CLIMATE IN BRAZIL

SUMMARY

Water erosion is one of the main degradation processes of tropical soils. In steepest areas with coffee cultivation, the erosion rates are intensified and could reach levels above the Soil Loss Tolerance Limits (T). Thus, the objective of this work was to evaluate the susceptibility to water erosion in steepest areas under predominant coffee cultivation using the Revised Universal Soil Loss Equation (RUSLE) and compare the results to T limit. The research was carried out at the Ribeirão José Lúcio subbasin located in Conceição do Rio Verde Municipality and the Ribeirão São Bento subbasin located in Cambuquira Municipality, both in South of Minas Gerais State, Brazil. The parameters involved in the RUSLE and T calculations were determined from the physical and edaphoclimatic characteristics of the subbasins. The total soil loss of the Ribeirão São Bento subbasin was 1,032 Mg year^{-1}, while the Ribeirão José Lúcio subbasin present an erosion rate of 5,014 Mg year^{-1} with 13.16% and 7.90% of the areas above the T limits, respectively.

We found the highest losses in steepest and exposed soil areas, which should be prioritized in the adoption of conservation management practices, seeking to minimize water erosion, and ensuring the long-term sustainability of agricultural production. The RUSLE model is a fast, simple, and inexpensive tool that contributes to the assessment of soil conservation in hydrographic subbasins.

Keywords: Erosion Modeling, Soil Conservation, Water Erosion, Revised Universal Soil Loss Equation, RUSLE.

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INTRODUCTION

Soil is one of the most important and most complex natural resources, but current developments (climate change, soil erosion, and urbanization) increasingly threaten this valuable resource (Ayer *et al.*, 2020; Spalevic *et al.*, 2020; Chalise *et al.*, 2019; Parsipour *et al.*, 2019; Curovic *et al.*, 2019.). Soils are essential for food production and various other ecosystem goods and services, including climate regulation and nutrient cycling (Greiner et al., 2017).

Soil degradation caused by erosion and rapid population increase is ranked among the most important environmental problems in the world (Khaledi Darvishan *et al*., 2019; Dimotta *et al*., 2017; Spalevic et al., 2016; Dimotta *et al*., 2016). Erosion is a key driver of land degradation, heavily affecting sustainable land management in various environments worldwide (Ouallali *et al.*, 2019; Tavares *et al.*, 2019; El Mouatassime *et al.*, 2019; Nikolic *et al.*, 2018; Spalevic, 2011). Water erosion is one of the main processes of tropical soil degradation and causes organic matter and nutrients losses, compromising the provision of soil ecosystem services (Olivetti *et al.*, 2015; Bertol *et al.*, 2007). According to Lal (2014), 1 billion hectares worldwide have already been affected by the erosion process, of which 70% are seriously committed to agricultural production. The worldwide annual rate of soil erosion from agricultural land ranges from 22 to 100 t ha^{-1}; declines in productivity as much as 15–30% annually (Morgan, 2005).

Estimating soil loss and identifying hotspot areas support combating soil degradation (Girmay *et al.*, 2020). Direct field measurements of soil erosion at permanent research or experimental stations using runoff plots with the known area, slope gradient slope length, and soil type could give reliable runoff and soil loss (Hurni *et al.*, 2010) for experimental purposes, however, it is costly, labor-intensive, and time-consuming (Alemayehu & Alamirew, 2012). Empirical-statistical models were developed and improved to evaluate and quantify water erosion (Hazbavi *et al.*, 2020; Amorim *et al.*, 2010; Spalevic, 1999). These models include the Universal Soil Loss Equation - USLE (Wischmeier and Smith, 1978) and the Revised Universal Soil Loss Equation - RUSLE (Renard *et al.*, 1997). RUSLE has a simple application and can be adapted to new geographical and edafoclimatic conditions. Moreover, combined this model with geographic information systems (GIS), it is possible to assess the spatial distribution of soil losses and identify areas most susceptible to erosion (Avanzi *et al.*, 2013). Furthermore, soil losses can be compared with the soil loss tolerance (T) limits, which represent the maximum erosion rate that allows sustainable agricultural production (Wischmeier and Smith, 1978).

Coffee is the main agricultural crop in southern Minas Gerais, with economic and social prominence. Coffee plantations are concentrated in steepest areas, which are more vulnerable to water erosion. However, few studies evaluate the dynamics of the erosive process in these areas. Thus, the objective of this work was to evaluate the susceptibility to water erosion in steepest areas under predominant coffee cultivation using the RUSLE and compare the results to the T limit.

MATERIAL AND METHODS

Study area

The research was carried out at the Ribeirão José Lúcio subbasin located in Conceição do Rio Verde Municipality, coordinated 473000 at 477000 m W and 7581000 at 7584000 m S, and the Ribeirão São Bento subbasin located in Cambuquira Municipality, coordinates 479000 to 484000 m W and 7570000 to 7575000 m S, Datum SIRGAS 2000, zone 23K UTM, both in southern Minas Gerais, at high altitudes (> 1000 m) in Serra da Mantiqueira, Brazil (Figure 1).

According to the Köppen system, the climate is classified as humid mesothermal, tropical of altitude subtype (Cwb), with an average temperature of 20° C and precipitation between 1,480 to 1,700 mm (Sparovek *et al.*, 2007). In both municipalities, coffee production is the main economic activity (Figure 1).

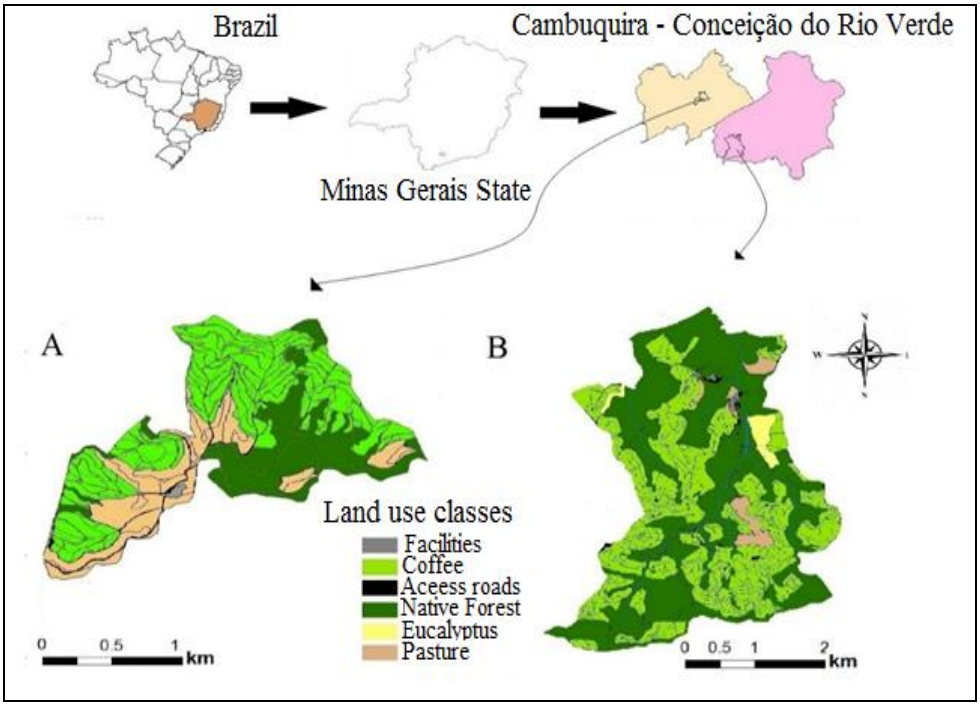

Figure 1: Location and land use and occupation maps in the Ribeirão São Bento (A) and Ribeirão José Lucio (B) sub-basins respectively in the municipalities of Cambuquira and Conceição do Rio Verde, southern of Minas Gerais, Brazil.

The elaboration of the land use map was made from the cartographic base and the crops mapped by Ipanema Agricola SA (Ipanema Coffees), and Landsat-8 Operational Land Imager (OLI) satellite images, bands 2, 3 and 4, corresponding to Orbit / Point 219/75 from Imaging Division (DIDGI) (INPE, 2019). The images were composting in ArcGIS 10.2 (ESRI, 2014), and the accuracy was verified in field surveys, with a 95% accuracy rate. Occupancy rates for land use classes are shown in Table 1.

Table 1: Land use and occupation classes in the Ribeirão São Bento and Ribeirão José Lúcio subbasins.

Ribeirão São Bento			**Ribeirão José Lúcio**		
Land use	Area (ha)	Area (%)	Land use	Area (ha)	Area (%)
Access roads	19.88	5.97	Access roads	80.20	5.91
Facilities	1.17	0.35	Facilities	18.18	1.22
Coffee	147.74	43.55	Coffee	543.94	40.26
Native forest	94.46	27.82	Native forest	669.01	49.35
Pasture	75.95	22.31	Pasture	34.05	2.52
	----	----	Eucalyptus	23.18	1.72
TOTAL	332.92	100	TOTAL	1355.39	100

The altitudes range from 893 to 1,339 m and 849 to 1,096 m for the Ribeirão José Lúcio and Ribeirão São Bento sub-basins, respectively (Fig. 2). The digital elevation model (DEM), with spatial resolution of 12.5 m, was made from the contours extracted from the topographic map of Varginha (IBGE, 1979) and São Lourenço (IBGE, 1971), with the ArcGis 10.2 tool to Raster (ESRI, 2014).

The slope map was generated (Figure 2) using the DEM by the ArcGis 10.2 Slope tool (ESRI, 2014). The relief units were classified according to EMBRAPA (2011), in flat (0-3%), slightly rolling (3-8%), rolling (8-20%), strongly rolling (20-45%) and mountainous (45-75%) relief. In both subbasins, there was a predominance of rolling and strongly rolling relief (Figure 2).

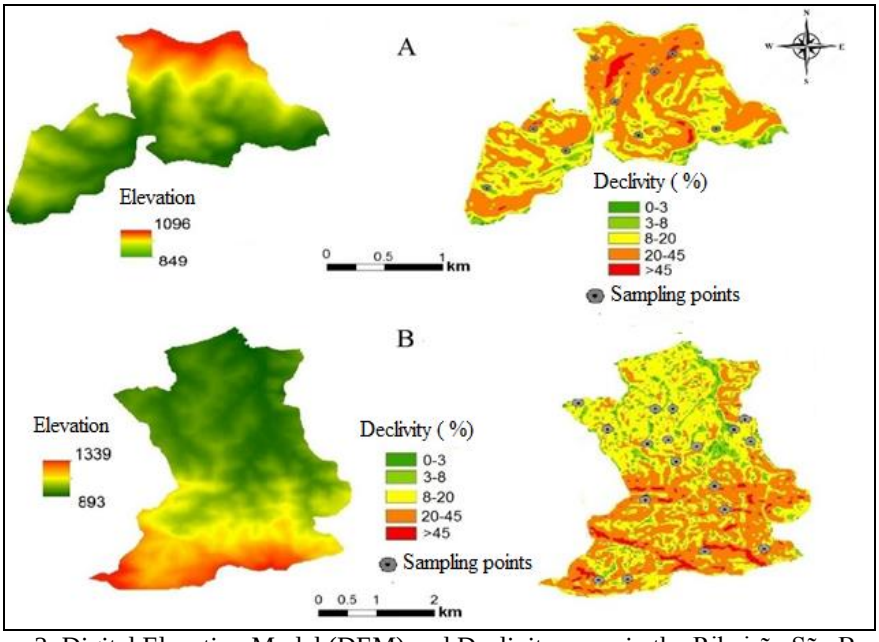

Figure 2: Digital Elevation Model (DEM) and Declivity maps in the Ribeirão São Bento (A) and Ribeirão José Lucio (B) subbasins in the respective Cambuquira and Conceição do Rio Verde Municipalities, Minas Gerais state, Brazil.

The digital soil map (Figure 3) was elaborated from the Minas Gerais State Soil Map in the ArcGis 10.2 (ESRI, 2014), on a scale of 1: 650.000 (UFV *et al.*, 2010) mutually with field surveys. We considered the relief as a base attribute of soil differentiation (McBratney *et al.*, 2003).

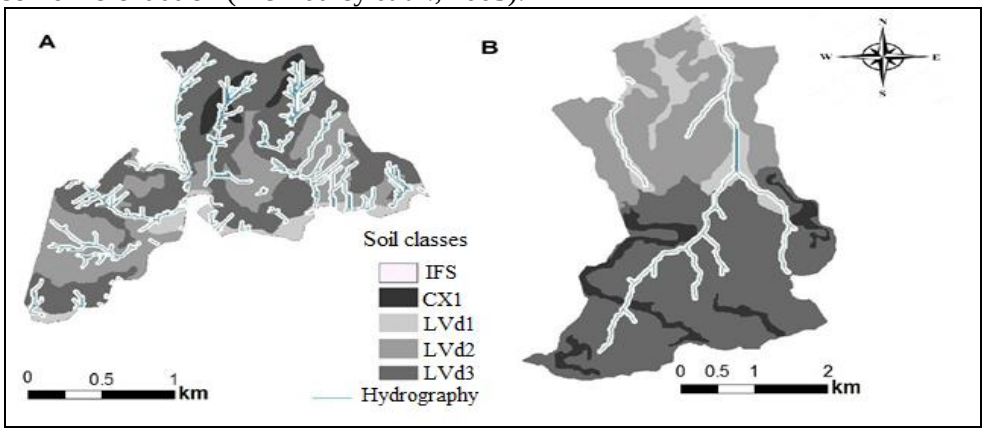

Figure 3: Digital soil maps: dystrophic red Latosol in a flat to slightly rolling relief (LVd1), dystrophic red Latosol in a rolling relief (LVd2), dystrophic red Latosol in a strongly rolling relief (LVd3), indiscriminate floodplain soils (IFS) and Haplic Cambisol (CX1) in the Ribeirão São Bento (A) and Ribeirão José Lucio (B) subbasins in the respective Cambuquira and Conceição do Rio Verde Municipalities, Minas Gerais State, Brazil.

The soils of the Ribeirão São Bento and Ribeirão José Lúcio sub-basins were classified as Latossolo Vermelho Distrófico in flat to slightly rolling reliefs (LVd1), rolling (LVd2), strongly rolling (LVd3); Cambissolo (CX1) in mountainous relief and indiscriminate lowland soils (IFS), according to the Brazilian Soil Classification System (Santos *et al.*, 2013) (Table 2).

Table 2: Soil classes, Ribeirão São Bento and Ribeirão José Lúcio subbasins.

Ribeirão São Bento			**Ribeirão José Lúcio**		
Soil classes	Area (ha)	Area (%)	Soil	Area (ha)	Area (%)
CX1	7.59	2.27	CX1	94.10	6.94
LVd1	20.86	6.26	LVd1	121.16	8.88
LVd2	108.11	32.47	LVd2	411.90	30.43
LVd3	151.56	45.55	LVd3	676.03	49.87
IFS	44.80	13.45	SIV	52.20	3.90
TOTAL	332.92	100	TOTAL	1355.39	100

CX1 = Haplic Cambisol; LVd1 = dystrophic red Latosol in a flat to slightly rolling relief; LVd2 = dystrophic red Latosol in a rolling relief; LVd3 = dystrophic red Latosol in a strongly rolling relief; IFS = indiscriminate floodplain soils.

Field sampling

Soil samples were collected based on land use and relief classes in 9 points at the Ribeirão José Lúcio subbasin and 18 points at the Ribeirão São Bento subbasin (Figure 2). We collect three types of samples on the surface (0 to 20 cm) and subsurface (20 to 40 cm) soil layers: disturb, undisturbed by the clod method, and undisturbed with a cylindrical sampler (volume 92.53 cm³ and depth 5 cm).

The following analyses were performed: particle size distribution with and without NaOH (Bouyoucos *et al.*, 1962 ; Blake *et al.*, 1986); organic matter (MO) by oxidation with $Na_2Cr_2O_7$ 2 mol L^{-1} + H_2SO_4 5 mol L^{-1}; pH with KCl and $CaCl_2$ - 1: 2.5 ratio; sum of exchangeable bases (SB); soil density by the volumetric ring method; cationic exchange capacity at pH 7.0 (CEC-T) and effective cationic exchange capacity (CEC-t); aluminum saturation index (m), remaining phosphorus (P-rem), exchangeable Ca-Mg-Al with 1 mol L^{-1} KCl extractor, H + Al with SMP extractor; available phosphorus (P) by the colorimetric method using ascorbic acid; base saturation index (V%); flocculation index and water dispersed clay by the pipette method (Zhang, 1997); aggregate stability with weighted average diameter (MPD) and geometric mean diameter (DMG) calculation by wet sieving method and soil porosity with total pore volume calculation EMBRAPA (2011). The soil permeability variable was obtained in the field, from three replicates for each soil class with a Mini Disk Decagon Devices infiltrometer adjusted for the suction rate of 2 cm Zhang (1997). The moist color was visually classified according to the Munsell (2012) classification.

Revised Universal Soil Loss Equation

Soil loss rates at the study areas were calculated by the RUSLE (Equation 1) (Renard *et al.*, 1997).

$$A = R \cdot K \cdot LS \cdot C \cdot P \qquad \text{(Equation 1)}$$

Where: A is a mean annual soil loss, Mg ha^{-1} $year^{-1}$; R is the rainfall erosivity factor, MJ mm ha^{-1} h^{-1} $year^{-1}$; K is the soil erodibility factor, Mg h MJ^{-1} mm^{-1}; LS is the topographic factor expressing slope and ramp length (dimensionless); C is the factor for land use and management (dimensionless), and P is the factor for conservation practices (dimensionless) (Wischmeier and Smith, 1978).

The R factor was obtained from the rainfall erosivity map for the southern Minas Gerais state, with values ranging in the two areas from 5,145 to 7,776 MJ mm ha^{-1} h^{-1} $year^{-1}$ with an average of 6,500 MJ mm ha^{-1} h^{-1} $year^{-1}$ (Aquino *et al.*, 2012). The K factor represents soil resistance to erosion. To Cambisol this parameter was calculated by the indirect method of Bouyoucos (1962) (Equation 2) and to the Latosols by the indirect method of Silva *et al.* (1999) (Equation 3).

$$K = [(\% \text{ sand} + \% \text{ silt}) / (\% \text{ clay})] /100 \qquad \text{(Equation 2)}$$

Where: K: average soil erodibility (Mg ha-1 MJ-1 mm-1); % sand, % silt, and % clay = percentages of the respective fractions of horizon A. The description and parameter values of Equation 2 are described in Table 3.

Table 3. Soil resistance to erosion (K) to Cambisol, according to Equation 2.

Factor		Ribeirão São Bento			
			% sand	% silt	% clay	K
K	Erodibility	CX1 Horizon A	70	11	19	0.043
		Ribeirão José Lúcio				
		CX1 Horizon A	67	16	17	0.048

Cambisol (CX1).

$$Y = -3{,}89 \times 10^{-2} + 5{,}11 \times 10^{-3} X_{14} - 1{,}25 \times 10^{-2} X_{15} + 5{,}41 \times 10^{-3} X_{16} - 7{,}27 \times 10^{-3} X_{18} + 5{,}33 \times 10^{-2} X_{33} + 3{,}21 \times 10^{-5} X_{34} - 5{,}66 \times 10^{-5} X_{36} + 8{,}33 \times 10^{-4} X_{2} - 1{,}17 \times 10^{-2} X_{4} + 1{,}53 \times 10^{-2} X_{13} \quad \text{(Equation 3)}$$

Note: The description and parameter values of Equation 3 are described in Table 4. The values of the variables were obtained based on soil samples collected from the native forest.

Table 4. Soil resistance to erosion (K) to Latosols.

Param.	**Description**	**Ribeirão José Lúcio**			**Ribeirão São Bento**		
		Soil Classes			Soil Classes		
		LV1	LVD2	LVD3	LVD1	LVD2	LVD3
Y	K	0.015	0.023	0.022	0.029	0.025	0.019
X_2	CEC-T pH 7.0 ($cmol_c\ kg^{-1}$)	6.490	9.170	8.238	13.315	11.086	5.120
X_4	pH = pH KCL - pH H_2O (dim)	-1.430	-0.710	-0.908	-0.820	-0.749	-0.753
X_{13}	KI relation(dim)	1.330	1.330	1.330	1.330	1.330	1.330
X_{14}	MSM Munsell (dim)	2.000	3.000	3.000	3.000	3.000	3.000
X_{15}	drainage (dim)	4.000	4.000	4.000	4.000	4.00V0	4.000
X_{16}	Structure degree (dim)	2.000	3.000	2.000	2.000	2.000	2.000
X_{18}	Structure shape (dim)	3.000	3.000	3.000	3.000	3.000	3.000
X_{33}	(TPV) ($dm^3\ dm^{-3}$)	0.628	0.608	0.607	0.600	0.619	0.645
X_{34}	Flocculation index (dim)	0.545	0.614	0.508	0.400	0.353	0.273
X_{36}	AS index ($G\ KG^{-1}$)	97.673	93.600	93.600	110.300	108.579	108.358

Dystrophic red Latosol in a flat to slightly rolling relief (LVd1), dystrophic red Latosol in a rolling relief (LVd2), dystrophic red Latosol in a strongly rolling relief (LVd3), cation exchange capacity (CEC), Dimensionless (dim), Moist Soil Matrix (MSM), total pore volume (TPV); Aggregate stability (AS).

The LS topographic factor was estimated according to Moore and Burch (1986) in the ArcGIS 10.2 (ESRI, 2014) (Equation 4) from the DEM using the

Raster Calculator tool. The model was efficient in determining LS, with higher factor values associated with steep slopes and more intense flows. The LS factor range from 0 to 238, with an average of 16.44 and 0 to 617, with an average of 7.28, for the Ribeirão São Bento and Ribeirão José Lúcio subbasins, respectively.

$$LS=\left(\text{Slope Length}\cdot\frac{12.5}{22.13}\right)^{0.4}\cdot\left(0.01745\cdot\frac{\text{Slope in Degree}}{0.0896}\right)^{1.4}\cdot 1.4 \qquad \text{(Equation 4)}$$

Where: LS = topographic factor (dimensionless); 12.5 = DEM cell size.

To determine the C and P factors, we consult the specialized literature. Areas with exposed soil present the highest C values, followed by eucalyptus cultivated down the hill, coffee, degraded pasture, facilities, indiscriminate floodplain soils, and native forest (Table 5). The higher P factor was found in degraded pasture and exposed soils, while the lowest value found in the native forest was due to the dense vegetation cover (0.01). Coffee presents a P factor of 0.50 due to conservationist practices.

Table 5. Land use and management (C) and conservation practice (P) factors for the Ribeirão São Bento and the Ribeirão José Lúcio subbasins.

Land use and occupation	Factor C	Source factor C	Factor P *
Coffee (3.95 x 0.55 m)	0.135	Prochnow 2005	0.50
Degraded pasture	0.100	Roose 1977	1.00
Native forest	0.001	Silva 2016	0.01
Floodplains soils	0.004	Oliveira 2007	0.00
Facilities	0.010	Lin 2011	0.00
Eucalyptus down the hill	0.300	Martins 2010	1.00
Exposed soil	1.000	----	1.00

*Valores de P obtidos de Bertoni e Lombardi Neto (2012) e Roose (1977).

Validation

The validation of soil loss estimates was done by monitoring the annual sediment transport, according to Beskow *et al.* (2009). For this purpose, data of total solids in water and respective flow monitored from 1997 to 2010 by two hydrosedimentological stations operated by the Minas Gerais Institute for Water Resources Management (IGAM), located in the municipalities of Cambuquira (MG 473138 W and 7581539 S), and Conceição do Rio Verde (MG, 490706 W and 7572704 S). Afterwards, the annual sediment transport was calculated considering the flow of the sub-basins and the daily flow of data obtained from the National Water Agency (ANA).

The annual sediment transported was compare with the Sediment Delivery Ratio (SDR), which represents the eroded soil fraction that reaches the water bodies. The SDR value is determined according to Equation 5 Vanoni (1975).

$$SDR=0.473\cdot(0{,}00386102*A)^{-0.125} \quad \text{(Equation 5)}$$

Where SDR is Sediment Delivery Ratio (%); A is basin drainage area (ha).

Soil Loss Tolerance (T)

The T was calculated by Equation 6 (Bertol and Almeida, 2000).

$$T = h \cdot r_a \cdot m \cdot p \cdot Ds \cdot 1000^{-1} \quad \text{(Equation 6)}$$

Where T is the soil loss tolerance (Mg ha^{-1} $year^{-1}$); h is the effective soil depth (cm), limited to 100 cm; r_a is the ratio that expresses, mutually, the effect of the textural relationship between the horizons B and A and the clay content of the horizon A; m expresses the organic matter content in the 0 - 20 cm soil depth; p is the soil permeability factor; Ds is the soil density (kg dm^{-3}); and 1.000 is the constant that expresses the time period required to wear away a soil layer of 1,000 mm thickness.

Latosols and Cambisols of the study area present an effective soil depth (h) of 1000 mm and 800 mm, respectively. The other parameters were determined according to Bertol and Almeida (2000), using the soil analyses results. Both subbasins present a r_a of 1 and an m and p of 0.7, with soil permeability classified as slow. Soil density of Latosols and Cambisols was, respectively, 1.23 kg dm^{-3} and 1.21 kg dm^{-3} for Ribeirão Jose Lucio and Ribeirão São Bento subbasins.

RESULTS AND DISCUSSION

The total soil loss of the Ribeirão São Bento subbasin was 1,032 Mg $year^{-1}$, while the Ribeirão José Lúcio subbasin presents a loss of 5,014 Mg $year^{-1}$. The sediment delivery ratio (SDR) was 0.045 and 0.38 indicating that 45% and 38% of eroded sediments in the respective Ribeirão São Bento and Ribeirão José Lúcio subbasins reach the water bodies. Thus, considering the SDR, the average soil loss estimated by RUSLE was 1.41 and 1.22 Mg $year^{-1}$ ha^{-1} (Table 6).

We found errors of 0.19 Mg $year^{-1}$ ha^{-1} (15.57%) and 0.25 Mg $year^{-1}$ ha^{-1} (19.82%) comparing RUSLE results with the annual sediment transported calculated using field data (Table 6).

According to Pandey (2007), errors smaller than 20% allow the validation of the water erosion models. Thus, the results generated by RUSLE illustrate the satisfactory efficiency of the method employed.

Table 6. Soil loss estimate by RUSLE, annual sediment delivery and estimate errors.

Subbasin	Soil loss rate (Mg ha^{-1} $year^{-1}$)	Annual sediment delivery (Mg ha^{-1} $year^{-1}$)	Error (Mg ha^{-1} $year^{-1}$ and %)
José Lúcio	1.41	1.22	0.19 (15.57)
São Bento	1.39	1.16	0,23 (19,82)

W_{yr} = Annual erosion; G_{yr} = real soil loss;

Areas with exposed soil and steeper slopes have the highest rates of soil loss in both sub-basins (Figure 4).

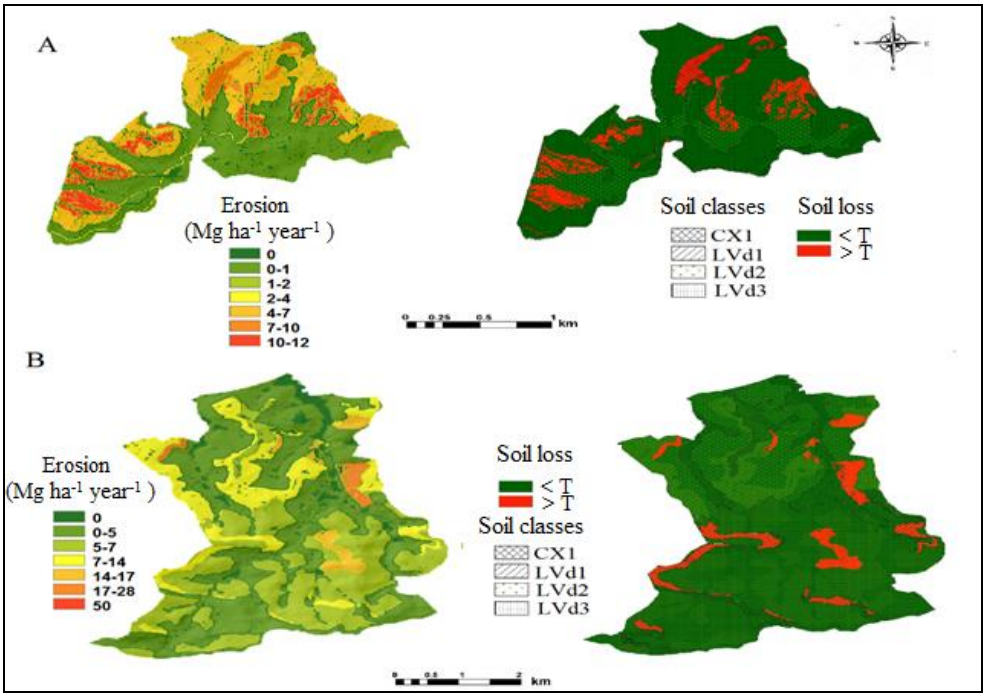

Figure 4: Soil loss rates and Soil Loss Tolerance (T) in the Ribeirão São Bento (A) and Ribeirão José Lucio (B) subbasins in the respective Cambuquira and Conceição do Rio Verde Municipalities, Minas Gerais State, Brazil.

Note: Dystrophic red Latosol in a flat to slightly rolling relief (LVd1), Dystrophic red Latosol in a rolling relief (LVd2), Dystrophic red Latosol in a strongly rolling relief (LVd3).

As expected, due to the greater fragility of Cambisols, the sediment generation rates in each class of land use showed that Cambisols are more susceptible to erosion compared to Latosols (Bertol and Almeida, 2000) (Table 7).

The soil loss rate estimated in the native forest was 0.01 Mg ha^{-1} $year^{-1}$, similar to Silva *et al.* (2007) that found soil loss rates range from 0.01 to 0.38 Mg

ha^{-1} year^{-1}, in a native forest at the Rio Grande do Sul State. The low losses in native forests are due to natural conservation and the protection offered to the soil by the canopy of dense vegetation and litter.

Coffee areas presented average soil loss rates of 4.50 and 5.71 Mg ha^{-1}year^{-1} for the Ribeirão São Bento and Ribeirão José Lucio subbasins, respectively. The highest rates of erosion in coffee were found in young areas, when soil cover by the canopy of coffee trees is still low (Carvalho *et al.*, 2007). The results obtained were lower than the values observed by Silva *et al.* (1999) (10.98 Mg ha^{-1} year) for Dystrophic Red-Yellow Latosol. These results are due to the conservation practices adopted in the coffee areas, with consequent lower P factor value (0.5).

Table 7. Soil losses by the land use and occupation classes in the Ribeirão São Bento and the Ribeirão José Lucio subbasins.

	Ribeirão São Bento			**Ribeirão José Lucio**		
Land use	Area (ha)	Area (%)	Soil loss Mg ha^{-1} year^{-1}	Area (ha)	Area (%)	Soil loss Mg ha^{-1} year^{-1}
Facilities	1.17	3.35	0.00	10.01	0.73	0.24
Coffee	147.74	43.55	4.50	538.94	39.76	5.71
Access roads	19.88	5.86	6.27	80.20	5.91	6.33
Native forest	94.46	27.84	0.10	669.01	49.35	0.24
Pasture	75.95	22.31	0.21	34.04	2.51	3.01
Eucalyptus	-	-	-	23.18	1.71	18.24
Total	332.92	100	-	1355.39	100	-

Eucalyptus showed the highest rate of soil loss among the land use classes (Table 7), due to the young age of the plants, which provides low canopy protection against erosion. The T limits determined for the Ribeirão São Bento and the Ribeirão José Lúcio subbasins were 8.3, 7.5, 7.1 and 6.7 Mg ha^{-1} year^{-1}, and 6.5, 8.5, 7.5 and 5.5 Mg ha^{-1}year^{-1} for the LVd1, LVd2, LVd3, and CX1, respectively. Ribeirão São Bento subbasin has 13.16% of the area with losses above T, while 7.9% of the Ribeirão José Lúcio subbasin area exceeded the T limits.

The T results obtained are below those found by Bertol and Almeida (2000) for Latosols from Santa Catarina State (10.62 to 12.50 Mg ha^{-1} year^{-1}) and São Paulo State (9.60 to 15.00 Mg ha^{-1} year^{-1}) according to Bertoni and Lombardi Neto (2012). This difference may be due to Bertol and Almeida (2000) method considering more attributes related to the soil formation factors in the T estimation.

Determining the T is quite difficult due to the difficulties in calculating soil formation rates. For this reason, soil properties, such as organic matter, water permeability in the soil and the textural relationship between horizons B and A,

which indirectly reflect the rates of soil formation, are used to define T. Conceptually, every soil has a limit T, which is related to your training rate. Thus, T calculations are complementary to water erosion estimates and allow a more accurate assessment of soil degradation status

Areas with soil losses above T should be prioritized in the adoption of conservation management practices, seeking to minimize water erosion, and ensuring the long-term sustainability of agricultural production. Better management practices such as terracing, level planting, and cover crops between the coffee lines could mitigate the erosion rates and decrease the runoff, consequently provide the conservation of watercourses, and improve the fertilizer use efficiency, which reduces the production costs (Bertoni and Lombardi Neto, 2012).

Considering the importance of coffee growing in high altitudes and steep areas in the south of Minas Gerais State, the results showed that the adoption of conservationist management practices provide low soil loss rates and contribute to the sustainability of coffee production. The studied subbasins presented distinct values of soil loss susceptibility but similar characteristics in the places most susceptible to erosion. The RUSLE model allowed the identification of areas with soil losses above the limits of T, especially in steep areas with coffee cultivation. Thus, it is an alternative tool for planning land use and management to promote sustainable agricultural systems.

CONCLUSIONS

Ribeirão São Bento and Ribeirão José Lúcio subbasins soil losses ranged from 0.01 to 28.45 Mg ha^{-1} $year^{-1}$, with an average of 1.41 and 1.22 Mg $year^{-1}$, respectively. The average soil loss in the coffee cultivation areas was 5.1 Mg ha^{-1} $year^{-1}$.

Revised Universal Soil Loss Equation modeling of water erosion showed higher losses rates in areas with steeper slopes and without conservation practices. The areas with soil loss above the tolerance limits should be a priority for the adoption of mitigation measures.

The RUSLE model is a fast, simple, and inexpensive tool that contributes to the assessment of soil conservation in hydrographic subbasins.

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ANALYSIS OF GENETIC VARIATION IN BREAD WHEAT BY GRAIN YIELD COMPONENTS

SUMMARY

The aim of this research was to evaluate the genetic variability of 18 wheat (*Triticum aestivum* L.) genotypes by quantitative agro-morphological traits, and to identify factors affecting the cultivars grain yield. A two years study (2017-2019) was conducted at the Experimental Field of the Agricultural University of Tirana (altitude of 40 m above the sea level, Latitude 41°24'6.14"N and Longitude 19°44'9.93"E). Plant material used, is part of the 270 accessions of the base collection of the Institute of Plant Genetic Resources. During the growing crop years, the accessions were evaluated for different quantitative characters and morphological characterization of the accessions was conducted according to international standards. ANOVA, principal components and cluster analysis were carried out involving nine quantitative traits, such as tiller capacity, plant height, spike length, number of spikelet's per spike, number of seeds per spikelet, number of seeds per spike, weight of seeds per spike, weight of 1000 seeds and grain yield.

According to PCA, three components exhibited about 75.51% of the variability within 18 wheat genotypes. Accessions were grouped into three major clusters based on Euclidean distance, suggesting that wheat genotypes with major level of dissimilarity between them were "Generozo" and "Ejesh". The results revealed that tiller capacity, number of seeds per spikelet, weight of seeds per spike/g and weight of 1000 seeds/g were the most important characters in differentiating the genotypes.

Wheat genotypes used in this study reacted differently in the two growing seasons, giving high results in most of the traits that contribute directly in grain yield during 2017/2018 crop year. The use of principal component and correlation coefficient analysis in the wheat germplasm, simplify dependable classification of bread wheat germplasm, the identification of the superior genotypes and their relation with bio morphological traits with possibility expenditure in future breeding programs.

Keywords: variability, traits, grain yield, PC analysis

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INTRODUCTION

Common wheat (*Triticum aestivum* L.) is among the most important cereals cultivated for human consumption in most parts of the world. The crop is used by more than one billion people and it is grown on approximately 220 million hectares of land worldwide, with a total annual production of about 729 million tons (FAO, 2017). It has been projected that the demand for wheat in the developing world will increase 60 percent by 2050 which is a sobering forecast considering global wheat yields have remained constant for more than a decade (Curtis, 2002). Wheat (*Triticum aestivum* L.) because of its nutritional value is one of the most important cultivated food crops in Albania, with 70 000 ha of cultivated area which contributes to 22.5% of the country's GDP providing the income base for most of the population and serves as an employment safety net (FAO, 2017).

Genetic variability is one of the factors important for plant breeding programs. The evaluation of genetic variability based on morphological characters especially those of economic interest might be used to select appropriate materials in breeding programs for crop improvement, because in classical breeding process determination of variability between varieties is based on a large number of morphological characteristics (Dos Santos et al., 2009; Bode et al., 2013). Wheat grain yield is the result of some important physiological traits occurring in the growth and mostly is determined by the number of spikes, the number of grains and grain weight. The importance of these yield traits to wheat grain yield depends on the growth stage and management type (Okuyama et al., 2004).

The correlation coefficients analyze is a reliable statistical method, which provides tool to quantify the associations among different traits and indicate whether the influence is reflected in the yield. The evaluation of phenotypic variability by multivariate analysis gives the possibility to include a large number of accessions and to identify the most suitable resources for special traits (Goel et al., 2015). Analysis of morphological characteristics gave a clear insight into the existing genotypes diversity that can be used as a parent lines for a new selection cycle.

The objective of the present study was to provide a clear assessment of the variability and to evaluate the interrelationships among yield contributing traits through characterization of 18 accessions of wheat (*Triticum aestivum* L.) germplasm, to identify genetically divergent wheat genotypes with desirable traits that can be used as donors in hybridization for grain yield and other yield contributing components.

MATERIAL AND METHODS

The study is based on the characterization of the diversity of 18 genotypes of bread wheat (*Triticum aestivum* L.), part of the base collection of Plant Genetic Resources Institute (Table 1, https://www.eurisco.ipk-gatersleben.de/apex/).

Experiment carried two replications during the autumn season 2017/2018 and 2018/2019 at the Experimental Station of Valias, Tiranë (altitude of 40 m above the sea level, Latitude 41°24'6.14"N and Longitude 19°44'9.93"E). Common wheat was sown at the end of November 2017 and 2018, and standard fertilization and cultivation practices for wheat were carried out. At the beginning of June 2018 and 2019, wheat ears were manually harvested with the aim of determining yield components.

During the each crop years, the accessions were evaluated for different characters of quantitative type as: tiller capacity (TC), plant height/cm (PH), spike length/cm (SL), number of spikelet per spike (NSpkSp), number of seeds per spikelet (NSSpk), number of seeds per spike (NSSp), weight of seeds per spike/g (WSSp), weight of 1000 seeds (W1000S) and grain yield/g (GY).

Table 1. List of the 18 *Triticum aestivum* L. genotypes object of the study

Genotype Nr.	Accession code	Accession name	Acquire date	Origin
G 1	AGB 2811	Generozo"E" (YG-3072)	19990722	GRC
G 2	AGB 2812	Yecora"E"(YG-6123)	19990722	GRC
G 3	AGB 2813	Nestos	19990722	GRC
G 4	AGB 2814	Aranthos (G-05322)	19990722	GRC
G 5	AGB 2815	Ejesh (G-84909)	19990722	GRC
G 6	AGB 2822	Verjina (G-84865)	19990722	GRC
G 7	AGB 2823	Dodoni	19990722	GRC
G 8	AGB 2825	Yecora "E"	19990722	GRC
G 9	AGB 2837	Golia	20010626	ALB
G 10	AGB 2839	Galves 87	20010626	UKR
G 11	AGB 3064	IKBA_05	20101125	ALB
G 12	AGB3066	Univers 1	20101125	ALB
G 13	AGB3067	Univers 2	20101125	ALB
G 14	AGB3068	Univers 3	20101125	ALB
G 15	AGB3069	Univers 4	20101125	ALB
G 16	AGB3070	Univers 5	20101125	ALB
G 17	AGB3071	Univers 6	20101125	ALB
G 18	AGB2838	Agimi	20010626	ALB

For weather condition the data from IGEWE (www.geo.edu.al) were used (Table 2). Vegetation season 2017/2018 was specific for bread wheat growing due to mild winter and a considerable rainfall during all the season in comparison to the vegetative season of 2018/2019. In the first-year higher precipitation was noted in spring time when wheat genotypes were in phase of intensive growth and needed higher amount of water.

Morphological characterization of the accessions was conducted according to international standards (IPGRI, 1985). Statistical tests were carried out by the Statistical Package for Social Sciences (version 21).

Table 2. Weather condition of the two growing seasons

	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total/Mean
2017/2018 crop season								
± T°C	8.8	9	10.3	12.9	19.8	22.1	25.4	13.816
±mm	126.4	70	210	200	2.5	150	100	122.7
2018/2019 crop season								
± T°C	9.9	7	9	14	18	17	26	14.414
±mm	100	100	45	0.3	100	137.4	0	68.957

RESULTS AND DISCUSSION

The genetic variability between 18 wheat genotypes conserved in the Gene Bank is assessed using different agronomic traits, especially those related to yield component. Basic statistic for quantitative traits (Table 3) were used and the estimated variation coefficient was high for agronomic traits as grain yield, number of seeds per spike, spike length, weight of seeds per spike and weight of 1000 seeds, similar with others authors (Sabaghina et al., 2014; Xhulaj et al., 2019). Wheat genotypes used in this study reacted differently in the two growing seasons, giving high results in most of the traits that contribute directly in grain yield during 2017/2018 crop year. During the growth year of 2018/2019 the wheat genotypes presented high values only for plant height trait (Table 3).

Accession AGB 2823 (Dodoni) during the first year of crop production, resulted with highest values measured in four traits as plant height (±111.6cm), weight of seeds per spike (±3.12g), number of seeds per spike (±80.2) and grain yield (±400g). This genotype presented lower values not only for the same traits in the second year (2018/2019) but also among the 18 genotypes had the lowest tiller capacity measured. Aranthos wheat cultivar (G4) represent the highest values measured in traits (during 2017/2018 crop year) as number of seeds per spikelet (±4), weight of 1000 seeds (±51.2g) and grain yield (400g).

The wheat genotype IKBA_05 (G11) resulted with the higher number of spikelet per spike (±20) and weight of seeds per spike (±2.1g) traits among the 18 common wheat genotypes during the 2019 season, meanwhile for the first year of crop growth it differs from the other genotypes for the NSSpk trait (±4). Grain yield is influenced by spike properties and the spikelet number plays a very important role in the wheat grain yield (Sabaghina et al., 2014).

Albanian cultivar Univers 1 presented low measured values for some characters in the first year of planting, as tiller capacity, weight of seeds per spike and weight of thousand seeds, while we see an improvement in the second year of production in compare with other wheat genotypes part of the study, for components such as grain yield (148g) and weight of thousand seeds (47.1g).The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

Table 3. Descriptive statistics and ANOVA of quantitative traits in 18 genotypes of bread wheat (*Triticum aestivum* L.) measured in two years of crop growth

	Bio-morphological traits																	
	±TC		±PH		±SL		±NSpkSp		±NSSpk		±WSSp		W 1000 S/g		±NSSp		GY/g/m²	
Nr.	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
G 1	2.4	2.5	87.3	102	9.37	6.7	21.4	15.6	3	3	2.2	0.9	42.8	30	56.8	33	250	15
G 2	2.6	2	84.2	92.5	12.24	8.88	19.6	15.8	3	2	2.26	1.16	49.7	35	47.8	29.6	320	50
G 3	2.4	2.7	91.1	102.2	9.31	8.58	20	17.6	3	3	2.24	1.28	39.3	35	64	44.4	300	47
G 4	2.3	2.5	96.4	96.4	10.64	8.95	22.8	16.2	4	3	2.52	0.82	51.2	23	61	27.2	400	138
G 5	2.5	2.7	106	135.5	6.65	9.2	19	17	3.4	3	2.54	2.08	42	39	75.8	65.4	330	110
G 6	2.4	2.1	99.4	114.3	8.46	7.83	20.6	15.2	4	3	2.54	0.9	47	34	58	27.4	310	48
G 7	2.8	2	111.6	111.6	11.2	8.15	20.2	16.8	3.5	3	3.12	1.88	49	40.2	80.2	45.6	400	56
G 8	2.4	2	92.4	85.8	9.1	10.11	20.2	16.4	3.4	3	2.02	0.28	45	20.1	47.8	10.2	200	28
G 9	2	2	72.5	71.2	8.5	6.15	21.4	15.4	3	3	2.26	0.66	34	30	57.8	27.6	170	53
G 10	3	2	103.6	122.6	11.1	9.44	22	18.2	3	3	2.66	1.14	44	31	66.4	35.8	190	58
G 11	2.4	2.7	102.3	115	10.15	8.73	16.2	20	4	3	2.61	2.1	47	44.1	66.4	40.8	300	145
G 12	2	2	101.7	101.4	13.72	8.8	23.4	15.8	2	3	1.42	1.62	30.1	47.1	38	44.6	73	148
G 13	2	2	73.5	78.6	11.32	9.39	22.6	20	3	3	1.44	0.98	35.2	25.9	50	48.4	200	120
G 14	2	2.3	71.7	101.9	7.1	9.16	20.4	17.8	2	3	1.96	1.06	34	25	55.8	43.6	68	55
G 15	2	3	93.5	105.5	9.54	8.69	19.8	18.4	2	3	1.46	1.3	31.1	44.2	18.6	59.4	150	95
G 16	2.2	2.5	83.3	93.7	10.92	6.7	22.6	16.6	3	3	1.84	1.62	41.3	30	56.4	52.6	132	83
G 17	2	2	73.5	117.3	12.19	8.21	23.2	17.6	3	3	1.88	0.7	39.1	29	69	27.8	135	64
G 18	2.4	2	88	92.2	11.4	8.9	22.2	13	4	2	2.72	0.74	39	32	72.4	28	190	41
Mean	2.32	2.27	90.66	102.2	10.16	8.476	20.9	16.8	3.1	2.8	2.21	1.17	41.2	33.1	57.9	38.4	228.7	75.2
Stdv	0.29	0.33	12.39	15.81	1.830	1.044	1.79	1.71	0.6	0.32	0.47	0.51	6.43	7.56	14.5	13.5	102.1	41.1
CV%	13	14.8	13.7	15.5	18.0	12.3	8.6	10.2	20.9	11.2	21.7	43.2	15.6	22.9	25.0	35.3	44.6	54.6
std.er	0.06	0.07	2.92	3.72	0.43	0.24	0.42	0.41	0.15	0.07	0.12	0.12	1.52	1.78	3.41	3.19	24.1	9.67
F-Stat	0.17766		5.93749		11.51572		49.43897		1.92876		38.82968		12.06041		17.39823		35.08276	
P-Val.	0.676NS		<.02021*		<.00176*		<.00001*		0.173NS		<.00001*		<.00142*		<.00019*		< .00001*	

TC - tiller capacity; PH - plant height/cm; SL - spike length/cm; NSpkS - number of spikelet per spike; NSSpk - number of seeds per spikelet; NSSp - number of seeds per spike; WSSp – weight of seeds per spike/g ,WS-weight of 1000 seeds/g and GY-grain yield/g; I-2017/2018 season; II-2018/2019 season. F ratio values significant at the P0.05 level of the probability (*); NS-non significant.

Despite genetic composition, also climate factors as rainfall and temperature plays an important role in determining crop growth and influence

grain yield. Rainfalls play a significant role in both vegetative and reproduction stages of plant growth. Other studies (Liu et al., 2003) identified that wheat yield and quality was affected by average rainfall during seed planting time, flowering and grain-filling time. Moisture stress during the flowering, pollination, and grain-filling stages is especially harmful to wheat crop. Temperature is a modifying factor in all stages of wheat development including germination, tillering, booting, ear emergence, anthesis and maturity since it can influence the rate of water supply and other substrates necessary for growth, but varies with plant species, variety and phenological stages (Wahid et al., 2007).

We observed that all our genotypes produced higher grain yield during the first season in compare to the second growing season. In terms of total precipitation during the entire growing season, there exist differences between the two years of crop growth where main rainfall at the level of ±127.7mm is observed during the first season (2017/2018) and a lower value of precipitation (±68.95mm) during 2018/2019 crop year (Table 2).

Differences exist also in the distribution of precipitation between the two growth stages that vegetative and reproductive within one season, followed with visible differences between two years of crop production. During 2018 season the vegetative stage was characterized by high precipitation (±135 mm), meanwhile during 2019 this stage was associated with a lower level of precipitation (±81.66 mm) with a difference of ±53.8mm of rainfall. Little differences is observed for the air temperature at the level of ±0.73°C between year's crop for the same stage.

Regarding reproductive stage of our wheat genotypes, during 2018 this phase was related with a level of ±117.5mm of precipitation in comparison with the second year (2018/2019) where low level of rainfall was recorded (±79.23 mm) during the same phase. Data observed (Table 2) reveal a difference of 1.93°C in air temperature between the two growing seasons. Results suggest that our wheat genotypes to produce higher grain yield need moderate rainfall and air temperature.

We observed that the highest grain yield level during 2017/2018 in compare to 2018/2019 was associated with the highest vegetative precipitation and air temperature, also with highest reproductive stage precipitation (Table 2).

Analysis of morphological quantitative characters: ANOVA analysis found the presence of significant differences between wheat genotypes for most of the bio-morphological characters at the probability of P 0.05 (Table 2). Significant differences are observed within the genotypes for plant height, spike length, number of spikelet's per spike, weight of 1000 seeds, number of seeds per spike and grain yield.

The results show that the genotypes with the highest grain yield as "Aranthos" cv. (G4), "Ejesh" (G5), "Dodoni" (G7) and IKBA_05 (G11) were those with high values in TC, WSSp. WS, and NSSpk (Figure 1). According to this analyze strong positive correlation is observed between grain yield and

NSSpk (0.623*); tiller capacity (0.524*); weight of seeds per spike (0.625*) and weight of 1000 seeds (0.680*).

Correlation coefficient analysis: The correlation analyze is useful to identify agronomic traits that are positively related with yield (Bode et al., 2013). According to the multivariate correlation analyze different traits exhibited positive relations between them (Table 4), as tiller capacity of the wheat genotypes with weight of seeds per spike (r=0.64) and weight of thousand seeds (r=0.59). Strong positive correlations are observed between weight of thousand seeds and weight of seeds per spike (r=0.7) and also between number of seeds per spike and weight of seeds per spike (r= 0.751). Strong negative correlations existed among NSpkSp traits and most of the other traits as PH (r=-0.21), TC (r=-0.34), WSSp (r=-0.41), WS (r=-0.46), and GY (r=-0.23), same results are reported from previous studies (Xhulaj et al. 2017).

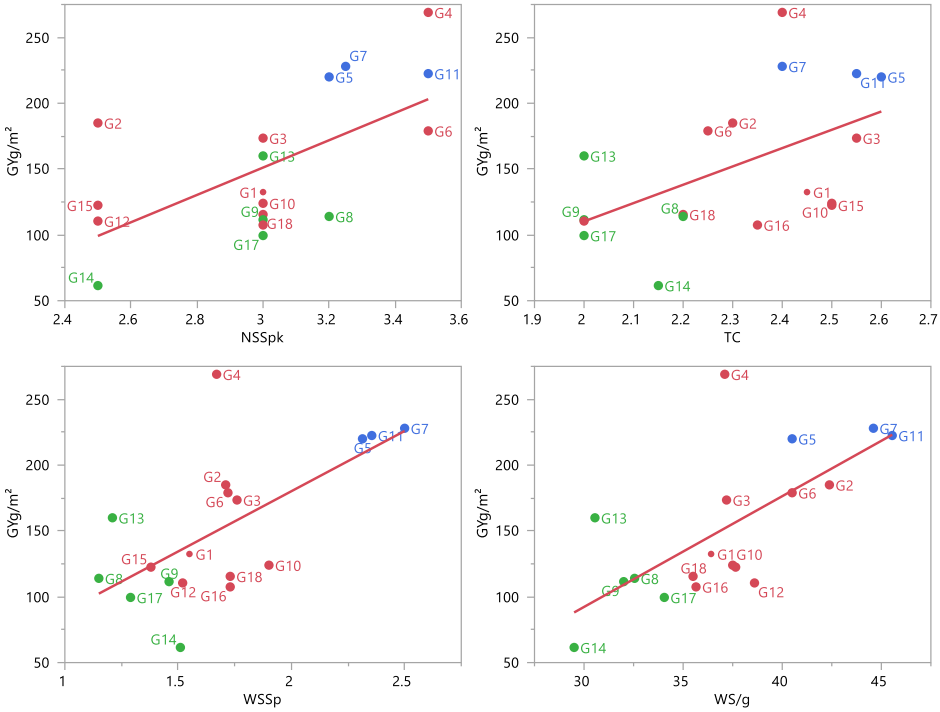

Figure 1: ANOVA analyses bivariate fit of grain yield by traits

Principal Component Analyses: Results of PCA suggest that 75.51% of the variability is exhibited among 18 common wheat genotypes by three components (Table 5) where the two first components influenced mostly the variability (PC1 48.82% and PC2 15.004% Figure 2). The variability presence within the first component was basically related with traits as weight of seeds per spike/g and weight of 1000 seeds/g, also by plant height/cm or grain yield, but poor variability in number of spikelet's per spike.

Table 4. Correlation matrix among the morphological traits (Pearson (n))

	TC	PH	SL	NSpkSp	NSSpk	WSSp	WS	NSSp	GY
TC	1.00	0.65	-0.23	-0.34	0.28	0.64	0.59	0.42	0.52
PH	0.65	1.00	0.05	-0.21	0.32	0.71	0.72	0.50	0.46
SL	-0.23	0.05	1.00	0.37	-0.21	-0.10	0.16	-0.20	0.05
NSpkSp	-0.34	-0.21	0.37	1.00	-0.16	-0.41	-0.46	0.01	-0.23
NSSpk	0.28	0.32	-0.21	-0.16	1.00	0.40	0.30	0.26	0.62
WSSp	0.64	0.71	-0.10	-0.41	0.40	1.00	0.79	0.75	0.62
WS	0.59	0.72	0.16	-0.46	0.30	0.79	1.00	0.32	0.68
NSSp	0.42	0.50	-0.20	0.01	0.26	0.75	0.32	1.00	0.35
GY	0.52	0.46	0.05	-0.23	0.62	0.62	0.68	0.35	1.00

TC - tiller capacity; PH - plant height/cm; SL - spike length/cm; NSpkS - number of spikelet per spike; NSSpk - number of seeds per spikelet; NSSp - number of seeds per spike; WSSp –weight of seeds per spike/g ,WS-weight of 1000 seeds/g and GY-grain yield/g.

This last trait followed by spike length strongly contributes at the genotype's variability in the second component, but this PC was very poor in number of seeds per spikelet and in tiller capacity. The third principal component exhibited positive effects for number of seeds per spike and number of spikelet's per spike. According to this analyze, the variables that effect most the variability within the three components is number of seeds per spike.

Table 5. Eigen values and % total variance for PCA in 18 wheat genotypes

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	4.39	1.35	1.05	0.96	0.52	0.37	0.18	0.11	0.03
Variab. (%)	48.8	15.0	11.6	10.7	5.78	4.14	2.03	1.32	0.43
Cumul. %	48.8	63.8	75.5	86.2	92.1	96.2	98.2	99.5	100
Eigenvectors									
PH	0.38	0.21	0.01	-0.23	0.33	-0.62	-0.25	-0.42	-0.10
SL	-0.06	0.79	-0.2	0.05	-0.14	-0.02	0.51	-0.11	0.03
NSpkSp	-0.21	0.47	0.60	0.07	0.33	0.09	-0.32	0.35	-0.12
NSSpk	0.26	-0.2	0.22	0.74	0.04	-0.41	0.29	0.20	0.12
WSSp	0.44	0.02	0.08	-0.17	-0.35	0.01	0.11	0.33	-0.73
WS	0.41	0.21	-0.4	-0.05	-0.12	-0.01	-0.38	0.52	0.46
NSSp	0.31	0.01	0.62	-0.29	-0.38	0.11	0.17	-0.20	0.45
GY	0.37	0.14	-0.04	0.47	-0.03	0.544	-0.32	-0.45	-0.12

In addition to cluster analysis, biplot (genotype by trait data) has been applied to explain the percentage variance associated with each principal component obtained by drawing a graph between Eigen values and Principal components number. The vector view of the biplot suggest a strong positive correlation among traits, as indicated by the small obtuse angles between their vectors, especially between weight of thousand-seeds of the genotypes and weight of seeds per spike. Also, this positive relation (r=cos 0= +1) is observed

through weight of seeds per spike and thousand-seed weight and number of seeds per spikelet.

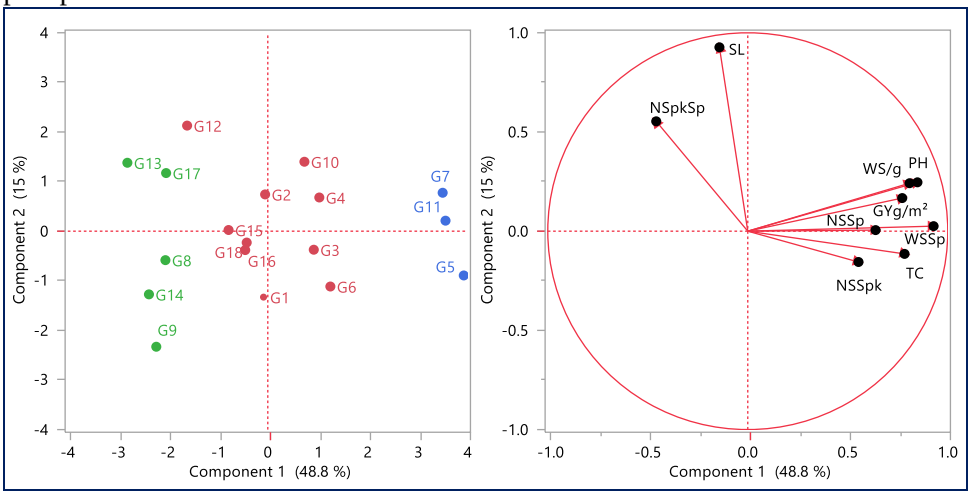

Figure 2: Principal component biplot of 18 wheat (*Triticum aestivum* L.) genotypes

The correlation between spike length and others traits as plant height, weight of thousand seeds, and finally grain yield, was near zero (r=cos 90= 0) as indicated by the near perpendicular vectors. The existence of a strong negative correlation of the vectors, indicated by the near angle of approximately 180 degrees is observed between spike length and number of seeds per spiklet, also among NSpkSp and NSSpk. The biplot (Figure 2) suggest that the best or the incompatible wheat genotypes in most of the traits, since they had the longest distance from the origin for the two principal components were Ejesh (G5), IKBA_05 (G11), Univers 1 (G12) and Univers 2 (G13). Therefore, it seems that for the first PC wheat genotypes (numbered at Figure 2) IKBA_05 (G11) and Ejesh (G5) have the highest values mostly for weight of seeds per spike, weight of thousand seeds and tiller capacity traits.

Cluster analysis: Genetic diversity among wheat germplasm was calculated after Agglomerative hierarchical clustering (AHC, Wards method, Figure 3). The 18 wheat genotypes were grouped in three major clusters. The first cluster include 10 bread wheat accessions, similar for different bio morphological traits (observed in two years of plants growth), the two wheat genotypes within this cluster with the lowest dissimilarity level observed are Generozo “E” (G1) and “Nestos” (G3). The 18 wheat genotypes were grouped in three major clusters. The first cluster include 10 bread wheat accessions, similar for different bio morphological traits (observed in two years of plants growth), the two wheat genotypes within this cluster with the lowest dissimilarity level observed are Generozo “E” (G1) and “Nestos” (G3).

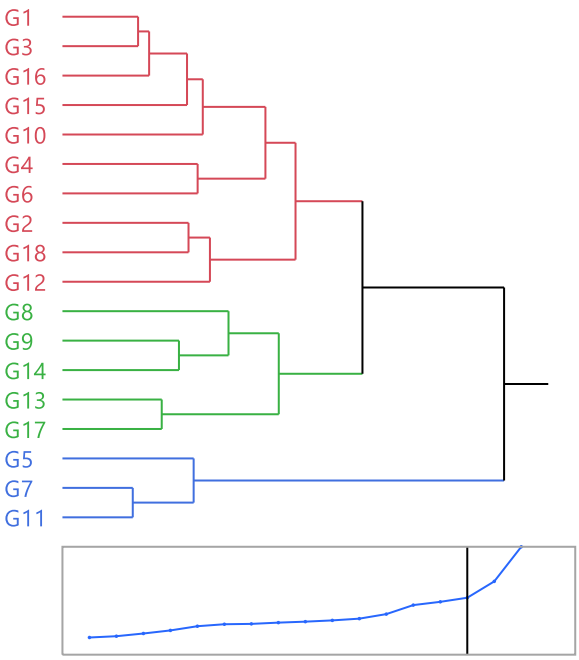

Figure 3: Dendrogram from cluster analysis of 18 bread wheat genotypes

The results suggest that the high similarity among them consists mostly in traits as tiller capacity, plant height/cm, spike length/cm, number of seeds per spikelet and weight of seeds per spike/g. These two accessions were joined from another genotype as "Univers 5" (G16) for similarity in weight of 1000 seeds/g and number of seeds per spike too. Within this cluster the two wheat genotypes with the highest level of dissimilarity observed are Generozo "E" (G1) and Yecora "E" (G2) basically for plant traits as height/cm, spike length/cm, weight of seeds per spike/g, weight of 1000 seeds/g, numbers of seeds per spike, grain yield g/m2.

The second cluster grouped five accessions whereas "Univers 2" (G13) and "Univers 6" (G17) presented the lowest distance among them, for similarity in tiller capacity and number of spikelets per spike. The third cluster included only three wheat genotypes, sub-clustered at this group with lowest level of dissimilarity observed not only within this cluster but also among the 18 wheat genotypes part of this research are "Dodoni" (G7) and "IKBA-05" (G11). These two accessions presented similarity in traits related to grain yield as weight of 1000 seeds/g, number of seeds per spikelet's, tiller capacity and spike length/cm.

The results reveal that the highest genetic distance exist between accessions part of the first cluster "Generozo E" (G1) and third cluster "Ejesh" (G5). These wheat genotypes were different in most of the agro bio-morphological traits as, plant height/cm, spike length/cm, weight of seeds per spike/g, weight of 1000 seeds/g, number of seeds per spike and grain yield g/m^2.

CONCLUSIONS

The results revealed that tiller capacity, number of seeds per spikelet, weight of seeds per spike/g and weight of 1000 seeds/g were the most important characters in differentiating the genotypes. Wheat genotypes used in this study reacted differently in the two growing seasons, giving high results in most of the traits that contribute directly in grain yield during 2017/2018 crop year. According to PCA, three components exhibited about 75.51% of the variability within 18 wheat genotypes. Accessions were grouped into three major clusters based on Euclidean distance, suggesting that wheat genotypes with major level of dissimilarity between them in most of the agro bio-morphological traits as, plant height/cm, spike length/cm, weight of seeds per spike/g, weight of 1000 seeds/g, number of seeds per spike and grain yield g/m2 were "Generozo" and "Ejesh". The results show that the genotypes with the highest grain yield as "Aranthos" cv. (G4), "Ejesh" (G5), "Dodoni" (G7) and IKBA_05 (G11) were those with high values in tillering capacity, weight of seeds per spike/g, weight of thousand seeds/g and number of seeds per spikelet's. According to ANOVA analyse strong positive correlation is observed between grain yield and NSSpk (0.62*); tiller capacity (0.52*); weight of seeds per spike (0.62*) and weight of 1000 seeds (0.68*). The use of principal component and correlation coefficient analysis in the wheat germplasm, simplify dependable classification of bread wheat germplasm, the identification of the superior genotypes and their relation with bio morphological traits with possibility expenditure in future breeding programs.

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BOKASHI USE IN THE PASSIONFRUIT (*Passiflora edulis* L.) GERMINATION AND INITIAL GROWTH

SUMMARY

The passionfruit has high relevance to Brazilian fruticulture, widely used in the food business. Its seedlings production, might attempt to the utilized substrate characteristics, its organic matter content, adequate nutrients concentration, good aeration, and pathogens agents' absence. Nowadays is increasing the use of renewable fertilizers in detriment to mineral ones, such as the bokashi and the organics compost in agriculture, although few studies are made to test how it affects different plants' metabolism. Due to this circumstance, more studies must test its effectiveness. The bokashi possesses several favourable characteristics as nutrients rapid release, soil structure maintenance, some pests, and disease prevention and control. This study used the bran rice bokashi and the organic compost aiming to verify its effect on passionfruit seedlings germination and initial growth in the tube at the greenhouse. The study area was in the fruticulture sector, on the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais (IFSULDEMINAS), Muzambinho Campus, Brazil. The treatments were different bran rice bokashi doses with organic compost: T1 (0%/100%, respectively bokashi/organic compost), T2 (2%/98%), T3 (4%/96%), T4 (8%/92%), T5 (16%/84%) and T6 (32%/68%). The adopted outline was the RBD, six treatments, and four repetitions, and eight tubes per portion. The obtained data submitted to statistical analysis, the treatments that stood out regarding the germination, were mainly the witness T1, T3, and T5. About the plants' growth treatments T4, T5, and T6; the T5 was the best treatment due to the high seeds germination and plant development rate on this experiment conditions.

Keywords: Alternative Substrates; Organic fertilizers; Seedlings production; Sustainable agriculture.

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INTRODUCTION

The passion fruit (*Passiflora edulis* L.) has as its birthplace tropical America and high relevance to the Brazilian fruticulture, once Brazil is the largest sour passionfruit producer and consumer in the world, being its fruits utilized as much to juice, sweet and sauces production as in natural consumption. Since this is a fruit culture in highlight into the national market, it very important to attempt on all cultivation aspects, from cultivar choose, seedlings production, crop handling until its harvest and commercialization, to thereby being optimized its production chain procedures. Although the passion fruit has a high capacity for a great production, the country presents low productivity compared to other producers in the world (Embrapa, 2016).

Silanaj et al. (2012) highlight that great fruit production depends on the seedling's quality, which is a result of the culture propagation methods. The most used technique for the passion fruit is per seed, due to its precocity in floral buttons production, which starts this process three to four months after its planting (Melo; Tavares, 2001). For being more advantageous, this method is the most applied by the fruit growers to the passion fruit seedlings production.

According to Melo and Tavares (2001), to a good passion fruit seedlings production, it must have to attempt to the utilized substrate characteristics, being important its organic matter content, adequate nutrients concentration, and good aeration, besides being pathogenic agents free. However, the commercial substrates generally do not provide organic sources mostly, beyond being costly to the producer. Thus, it is indispensable the creation and test of new quality and unexpansive mechanisms to seedlings production.

Nowadays we are recording increasing of the studies that are using alternative fertilizers such as the bokashi and organic compost in agriculture (Lense *et al.* 2020; Goelzer *et al.* 2019; Santos *et al.*, 2019; Pinto, 2018; Silva, 2016; Jaramillo-López *et al.*, 2015; Blecic *et al.*, 2014; Sorace, 2011; Cavalcante, 2010; Hafle *et al.*, 2009;). Although the bokashi use in tropical fruits as the passion fruit it is not something so explored in the literature.

The composts are alternative fertilizers, providing several advantages since they are biodegradable, generally result from plants and animals remain. They promote the soil nutrients cycling, the O.M. increase (Karimuna *et al*., 2016), and a favorable environment to biological agents that has pests and disease control-action. Moreover, a healthy environment generates healthy individuals, as in the trophobiosis theory (Rivera, 2014).

The bokashi is an organic fertilizer prepared from the fermentation promoted by the bacteria and other organisms found in an inoculant, the Effective Microorganisms - EM (Jaramillo-López *et al.*, 2015). Other elements can compound this fertilizer as wheat, rice, and corn bran, enriched with bones flour and animal manure as for poultry, being the bran most rich in nitrogen (Pinto, 2018). This composition varies according to the culture necessity and also with the available materials in the property (Jaramillo-López *et al.*, 2015; Rivera, 2014).

Yamada and Xu (2001) and Jaramillo-López *et al.* (2015), stand out greatly properties found out on fermented organic fertilizers that incremental EM, as the presence of soil and plants benefit organism, highlights the *Lactobacillus* spp. and the *Actinomycetes*, beyond nutrients and organic composts as aminoacidic, organic acids, macro, and micronutrients in small percentages. The authors also emphasize the organic fertilizer quality depends on initial preparation water quantity, organic carbon supply, and energy sources, besides the microbiological inoculant utilized.

When comparing nutrients sources, mineral fertilizing has as the advantage of its concentration, which is larger than the organic. Now the organic, according to Cavalcante (2010) and Jaramillo-López *et al.* (2015), possess as main advantages the soil structure maintenance, nutrients balanced release, supply charges for soil cationic exchange capacity (CEC) and organic matter (O.M.) incorporation, predators' microorganisms of pests and plant disease causal agents, besides supporting the root growth.

Another point that favors the organic sources' use is the mineral fertilizes rise price, due to this product importation and the constant dollar appreciation against the real, the unit of currency in Brazil. With organic sources utilization to the fertilizers and subtracts production to the property, the producer will have more profitability on final product sale, already his production costs will reduce, and he can use his property residues or from an agribusiness having the input with a reduced value, besides the environmental coverage, ruled in the organic residues reuse and recycling (Jaramillo-López *et al.*, 2015; Pinto, 2018). Popovic *et al.* (2020), for example, had as the objective of their study, in Podgorica vineyard region; the mineral fertilizers reduce use, substituting part of them by organic sources. They obtained good results for grape production, improving the use of mineral fertilizers and reducing the chance of environmental pollution from excessive utilization.

According to Ishimura (2004), the bokashi possess several favorable characteristics on the agriculture, once afford rapid nutrients release, assists on soil physics aspects, as its structure maintenance, besides also support in some diseases and pest's prevention and control, once it has a microorganism's broad range, introduced with the EM utilization, prepared from organism's virgin forest collected.

The passionfruit producers have a strong need to obtain low-cost seedlings than the alternative substrates use with security phytosanitary characteristics become essential. We aimed to study the bran rice bokashi effect in the passionfruit seedlings' germination and initial development from seeding to the point to send to the field in greenhouse conditions.

MATERIAL AND METHODS

The experiment was greenhouse conduced at Instituto Federal de Educação Ciência e Tecnologia do Sul de Minas Gerais, fruticulture sector, Muzambinho

campus, Brazil, whose coordinates are, latitude: 21º20'59,94''S and longitude: 46º31'34,82''W, with 1013 meters of mean altitude (Aparecido *et al.*, 2014).

The bokashi's treatment doses were adapted from Silva (2016) and Manoel *et al.* (2005) studies. The fruticulture sector produced the bokashi and substrate compounds. The bokashi obtained from two liters of EM (0.2 liters of concentrate EM + 0.2 liters of cane molasses + 1.6 liters of water without chlorine) added to 20 liters of bran rice and closed packed conditioned to its anaerobic production. The substrate was an organic compost, originated from leaves, and grasses remain with bovine manure, to achieve the relation C/N equal to 30/1, aerobically composted, after sifted, and added 20% in volume of wood shavings, thereon, steam-treated at 100ºC by at least one hour, for the substrate be out of pathogen agents.

The utilized seeds were obtained from commercial fruits, put to dry in the shade on environmental temperature per 24 hours into an absorbent paper to take off all the moisture and its mucilage. The seeds were selected and planted two to each tube. The selection takes on account the mature seeds that present a darker color and the larger ones because they have the largest nutrients reserves for their initial development.

The randomized block design (RBD) was the used one, with six treatments. The percentages utilized were detailed in Table 1, divided into four blocks at random, and each portion with eight tubes of 120 cm^3.

The Bokashi handoff by previous sifting hereafter weighed and measured the volumes of the Bokashi and the substrate. After the density of each compound verified, were established the proportions to compose the treatments in percentages (Table 1).

Table 1: Treatments adopted in the present work in percentages combining the substrate and the barn rice bokashi. IFSULDEMINAS, Muzambinho municipality, Minas Gerais state, Brazil 2019.

Treatments	**Bokashi**	**Substrate**
T1	0%	100%
T2	2%	98%
T3	4%	96%
T4	8%	92%
T5	16%	84%
T6	32%	68%

The watering was daily having the care of use water without chlorine to have no interference in the microorganism's population presents in the bokashi and consequently in the substrate, once that sought to verify their action in the seedling's emergence and initial growth. The seedlings thinning was realized

when they were with about 0,05 to 0,08 meters high, leaving only the more vigorous seedlings on each tube.

The realized phytometric evaluations were emerged seedlings number evaluated weekly, count until its population stabilization, being the count initialized fifteen days after the seeding (DAS), and the seedlings high measurement, from 45 DAS were realized weekly measurements until 60 DAS, being considered as useful installment the five marked central seedlings. In the emerged seedlings count was taken into account that each tube has just one seedling.

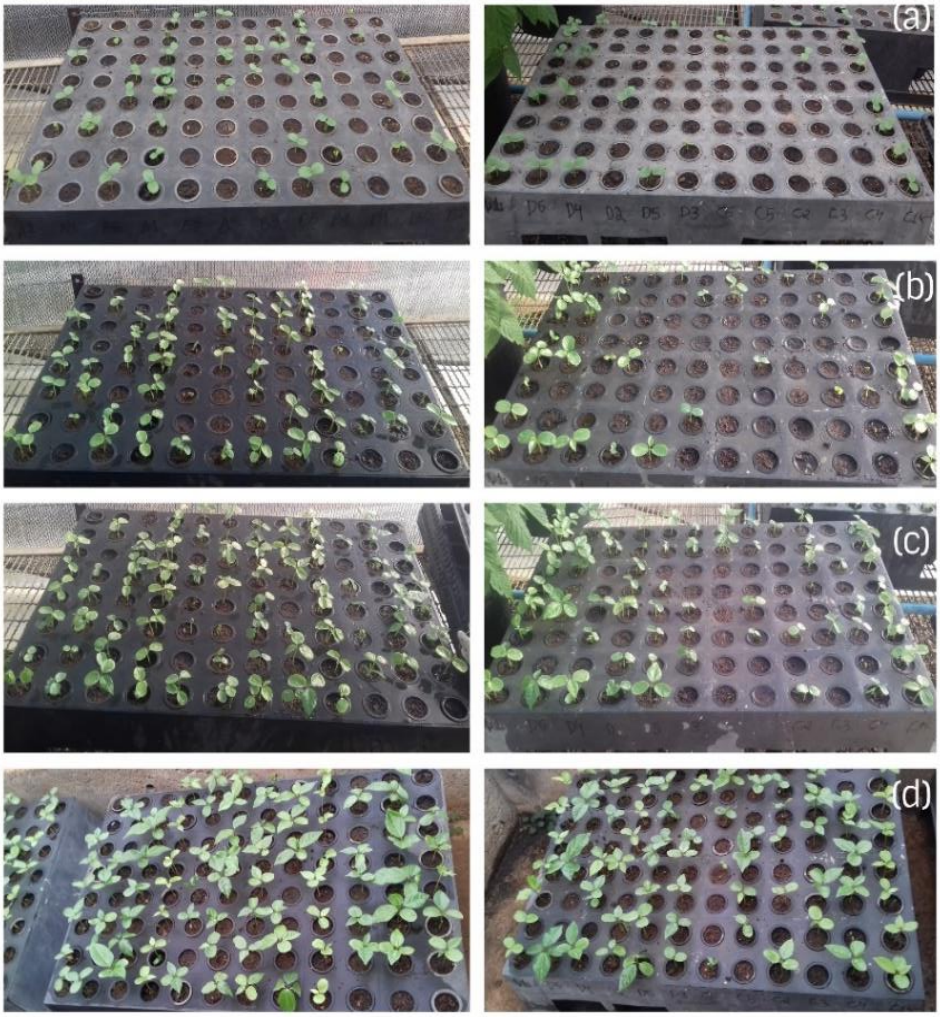

Figure 1a: Passionfruit germination and initial growth in barn rice bokashi and organic compost on each evaluation day. IFSULDEMINAS, Muzambinho municipality, Brazil.

Figure 1b: Passionfruit germination and initial growth in barn rice bokashi and organic compost on each evaluation day. IFSULDEMINAS, Muzambinho municipality, Brazil.
*(a) 15 DAS; (b) 21 DAS; (c) 28 DAS; (d) 35 DAS; (e) 42 DAS; (f) 49 DAS; (g) 56 DAS.

For the statistical analyses, the germination data transformed into percentages. There was no need for pests and disease control once there was no incidence in the passionfruit seedlings during the experiment.

The Tukey and statistical analysis of variance (ANOVA) test were employed to analyze the response variables at a 5% of probability through the statistical software R (R Core Team, 2020).

RESULTS AND DISCUSSION

As for the parameter emerged seedlings percentage, the T1 was superior to all other treatments on 21 DAS, meantime on 42 DAS, the treatments T3 and T5 do not differ statically of T1. The hypothesis is that the action of the bokashi microorganisms initially utilizes the nutrients, and only after slowly is release them to the plants, which differs from when it is only the substrate, so the seeds

that first emerged were the witness treatment (Figure 2). Such a hypothesis corroborated in Figure 3 that is self-evident on 60 DAS T4, T5, and T6 show themselves more efficient regarding the growing than the other treatments, probably the moment where that these nutrients are readily available to the seedling emerged absorb and grow.

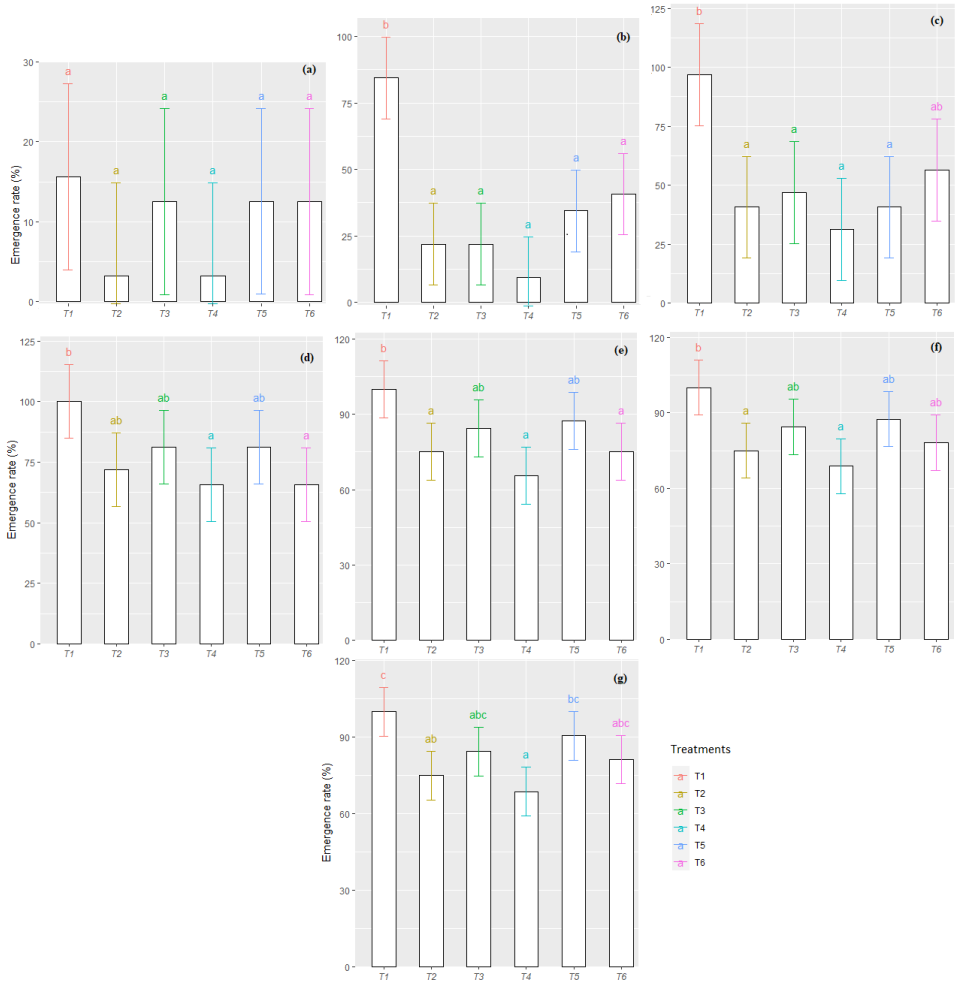

Figure 2: Passionfruit seedling's germination mean percentages and its respective treatment. IFSULDEMINAS, Muzambinho municipality, Minas Gerais state, Brazil, 2019.

Means followed by the same letter in the vertical has no difference among each other in the Tukey statistical test at 5% of significance. (a) 15 Days After Seeding (DAS); (b) 21 DAS; (c) 28 DAS; (d) 35 DAS; (e) 42 DAS; (f) 49 DAS; (g) 56 DAS.

In Santos *et al.* (2019) study, the bokashi increasing doses used in the substrate for the transplant of *Campomanesia adamantium* (Cambess.) O. Berg, a Cerrado Myrtaceae native fruit, was observed that the mortality could reach 100% of the seedlings when the bokashi content increased until the dose 20 g kg^{-1} of soil. In the case of this study of initial growth, was observed that the seeds in the substrate with bokashi took longer time to germinate, while the standard substrate had a greater germination index (Figure 2). Differently from what was observed by Santos *et al.* (2019), the used doses at the experiment do not cause passionfruit seedling death.

When in the first 28 DAS was observed that one of the treatments that added bokashi equalize the emergency to the witness but yet not differ from the other treatments. On the following evaluation days, the same response has been found in the bokashi treatments not being superior then the witness in any of them. Although this fact, we saw a tendency for treatments T5 to have greatest results for differing from the treatments with lower germination rates.

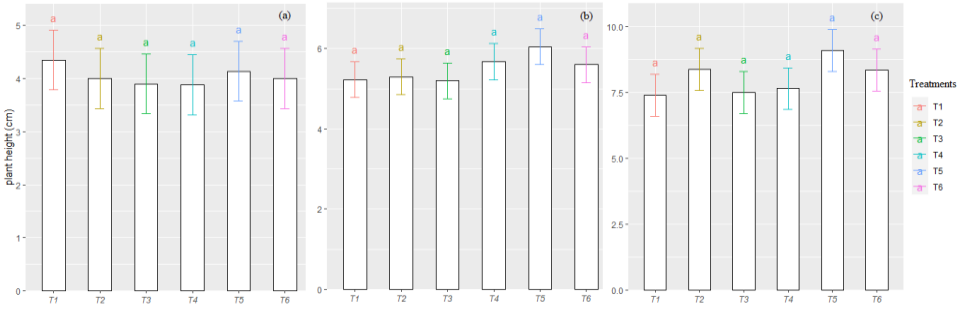

Figure 3: Passionfruit seedling's height mean values and its respective treatment. IFSULDEMINAS, Muzambinho municipality, Minas Gerais state, Brazil, 2019.

Means followed by the same letter in the vertical has no difference among each other in the Tukey statistical test at 5% of significance. (a) 45 Days After Seeding (DAS); (b) 53 DAS; (c) 60 DAS.

When performed the ANOVA test for plant height on 45 days after seeding, there is no difference among the treatments. In 53 and 60 DAS, the T5 (p value<0.05; 0.0135 and 0.00527 respectively) was superior to the other treatments when compared to the witness. Jaramillo-López *et al*. (2015), in their study of impacts of Bokashi on survival and growth rates of *Pinus pseudostrobus* in community reforestation in Mexico, found out that the trees that received Bokashi were significantly taller than those that not, what occurred here too. Another positive effect that Jaramillo-López *et al*. (2015) found is that the survival rate of the seedlings increased. SILVA *et al.* (2018), also observed greater values to plant height in the beet culture when added bokashi.

Although the ANOVA test pointed out the best treatment for the T5, in the Tukey test analysis, no difference reached out, regardless of the day (Figure 3).

Sorace (2011), at her alternative substrates to the potted chrysanthemum culture study, declares that the use of substrates with mineral fertilizers added to bokashi or humus increases the culture vegetative and the reproductive development, beyond the bokashi be better than the hummus since enhancing the inflorescences number. When we compare this study to the data obtained from this work, we can verify a tendency that the treatments that added bokashi present better results concerning its growing, mostly on 53 and 60 DAS the ANOVA test indicated that the T5 had the best response in improving seedling´s growth in comparison to the witness. Similar to what Oladele (2015) studies found, higher and vigorous plants, possibly because of the aid of nutrients uptake and water acquisition, in Akure Ondo State, Nigeria. The author inoculated arbuscular mycorrhizal fungi (*Glomus mossae*) on cacao (*Theobroma cacao*) seedlings. These arbuscular mycorrhizal fungi can be found on the EM to bokashi production because they live in virgin forests and almost all soils.

Hafle *et al.* (2009), on his papaya seedlings production study, observed that the bokashi at 60 DAS jointly with the seaweed powder (*lithothamnium*), had a significate effect as for the aerial part growth, the length had an upward linear growth according to the bokashi percentage increment into the substrate from 0 to 10%. The same relation observed in this present study on ANOVA test, that the superior bokashi doses afforded passionfruit seedlings higher aerial part growth when at 53 and 60 DAS, then the best treatment to this parameter is T5.

Manoel *et al.* (2005) on his different substrates to Arabic coffee cultivar Obatã (*Coffea arabica* cv. Obatã) seedlings study obtained the greater height values to the treatments with bokashi higher contents (10%), produced by anaerobic form, intermediate values (5%), from aerobic bokashi production, and with the bovine manure added to conventional fertilizer. Although no significant difference has been found from the other percentages of substrates with bokashi, the treatments with organic sources achieved the best performance. In this study, we obtained significative differences among the seedlings at 53 and 60 DAS on ANOVA test, when it is used the larger bokashi percentages in the treatments T5 (16 % of bokashi), differing statistically from T1, T2, T3, T4 and T6 (0, 2, 4, 8 and 32% of bokashi).

Goelzer *et al.* (2019), studying the *Campomanesia adamantium* (Cambess.) O. Berg initial growth observed that the Fertbokashi® influences positively in the stem dry matter having its maximum point next of 0,5 L ha^{-1}, also demonstrates itself positive in the relation between aerial part and radicular dry material tending to a positive linear function. Nonetheless, the applied Fertbokashi® doses barely influenced the guavira initial growth. However, in the present experiment, corroborate in parts on what found out in their study, we have observed significative influences in the initial growth fruitful tree here aborded, differing from the other treatments at 53 and 60 DAS by ANOVA test at 5% of significance. Karimuna *et al.* (2016) observed on their study that the bokashi improved the agronomic characteristics of peanut and maize in nutrient poor soils

in Indonesia demonstrating a potential use of this natural fertilizer in other cultures too.

CONCLUSIONS

The treatments that stood out for the germination were the witness (T1), in the beginning, and posteriorly the T3, T5 and the T6 equalized to the witness, however only the T5 did not demonstrate equivalence to the treatments with low germination percentages.

As for the plants growing, the treatment T5 were superiors to the others at 53 and 60 DAS in ANOVA test ($p<0.05$), meanwhile at Tukey statistical test at 5% of significance has no difference among the treatments.

The treatment T5 (16% Bokashi) was the one whose passionfruit seedlings growing were significatively greater on ANOVA test. Thus, this would be a treatment recommended to a seedling passionfruit grower.

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INFLUENCE OF NUTRITION ON PRODUCTIVITY AND CHEMICAL COMPOSITION OF KHORASAN WHEAT - *Triticum turgidum* L. ssp. *turanicum Jakubz*

SUMMARY

Khorasan wheat (*Triticum turgidum* L. ssp. *turanicum Jakubz*.) the white awn population is the optional type of a high-energy grain. This study aimed to examine the influence of plants' mineral nutrition on the chemical composition of Khorasan grain. The mineral diet used in the supplementary plant nutrition affected the total amount of dry matter and the chemical composition of wheat grains, increasing its nutritional value. At the time of harvest, Khorasan wheat grains contained 89.74% of dry matter, i.e. 10.26% of water, while in the variants with additional nutrition had less dry matter 86.90% and more water, 13.10%, which indicates an extension of the vegetation period of plants in conditions of enhanced mineral nutrition. Mineral nutrition, which primarily increased the amount of nitrogen, statistically significantly affected protein synthesis in plants. Khorasan wheat grains had 14.94% of total proteins in the control and 15.2% in variants with NPP nutrition. Plant nutrition did not have a greater impact on the synthesis of oils in the grains, starch, NFE, and mineral salts content. The supplementary nutrition affected the total amount of dry matter and chemical composition of wheat grain increasing its nutritional value.

Keywords: Khorasan wheat, supplementary mineral nutrition, the chemical composition of grain

INTRODUCTION

Khorasan wheat (*Triticum turgidum* L. ssp. *turanicum Jakubz*.) has been grown thousands of years ago in the area of ancient civilizations, the Middle East, and ancient Egypt. This ancient type of wheat was cultivated in the area of Big Khorasan (Northeastern Iran area of and neighboring countries) 6,000 years ago.

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Khorasan wheat was most likely formed by the spontaneous crossing of durum and field wheat. It is very close to durum wheat (AABB genome) in terms of genetic and morphological characteristics. In terms of nutritional value, it is superior to soft wheat and is a high-energy grain. Khorasan wheat (kamut) has very large grains of great nutritional value. Khorasan wheat is richer in oils, vitamins, and mineral salts. The increased content of monosaccharides gives the grain a sweet taste, so kamut is also called "sweet wheat". The flour obtained by grinding of these grains is added to wheat grains, in different proportions, for making pasta and bakery products of higher nutritional and energy value. These food products are suitable for the diet of more physically active people and people allergic to gluten because it contains significantly less gluten than wheat grains according to the research by the International Association for Allergies in Food (IFAA) (Ikanović et al., 2014).

In the diet of domestic animals, bran is used as a concentrated fodder, straw serves as a mat or as a raw material in further processing. Compared to soft wheat, kamut is more tolerant to drought and pests and has a higher coefficient of utilization of plant assimilates from the soil. Good production and biological properties have included this wheat in the system of organic farming, and the growing demand for food products has also affected the increase in the areas under this crop. In terms of average grain yields, Khorasan wheat is competitive, so it can be interesting for the system of conventional agriculture (Glamočlija et al., 2015; Ikanović, Popović, 2020).

The grain of this wheat is curved in the middle and larger than the classic varieties of wheat. Khorasan wheat is used to make good bread, toast, cakes, snacks, and other bakery products. Although lately, this ancient cereal has been attracting more and more attention in Serbian area little who is informed about it. In Western countries, Khorasan has become an unavoidable part of the daily menu, especially because it suits people who are sensitive to gluten. Kamut contains gluten, which is well tolerated by people who are sensitive to gluten from common wheat. Studies have shown that a large percentage of sensitivities be can reduced sensitivity consuming Khorasan wheat. Kamut grain is valued by consumers as a grain with high energy values, easy digestion, nutty taste, and firm structure. It contains more proteins, amino acids, selenium, and vitamin E than today's wheat varieties and contains essential minerals such as magnesium and zinc. It contains essential vitamins E and A, and is a good source of B vitamins. Also, it contains calcium, iron, magnesium, potassium, sodium, and zinc. Kamut provides fibber, carbohydrates, and essential macronutrients. Proteins play a significant role in strengthening tissues, and also help in oxygen transport, while fibbers lower cholesterol and affect the maintenance of the healthy digestive system. Kamut is a source of minerals selenium and manganese, which have an antioxidant effect. The grains consist of water, organic, and mineral substances. Among organic substances, the main ingredients are nitrogen-free extractives (NFE) i.e. starch and partially sugars, and their content varies from 50-70% and even more, and especially in hulled rice up to 75%,

followed by crude proteins, which range from 8-18 % and over it, and cellulose from 2-11%. The water content in the dry grain is around 14-15%. Of the nitrogen-free extractives, the grains contain the starch mostly, which is found in the endosperm and makes up about 80% of all carbohydrates. The rest falls on sugars, primarily cane sugar, which is primarily found in germ, about 1.5% of the weight of the grain. There is no starch in the germ. The oil content in the grain of cereals ranges from 2-4%. The oil is found primarily in germ, about 14% in wheat, 12.4% in rye, up to 26% in oats, and 20% in millet. Minerals - ash - are found mainly in the grain husk. In complex grinding, most of the minerals go into the bran, and the finer the grind is done, the less mineral matter exists in the flour. The main component of cereals ash is phosphorus, about 50%, then potassium, about 30%, magnesium, about 12%, and there is little calcium, about 2.8%. Cellulose is also found mostly in the shells of grains, and especially in husky forms and cell walls. The content of cellulose is higher in small grains than in large ones. In addition to the above ingredients, the grain also contains enzymes and vitamins. Enzymes play a significant role in the germination of grains, and in the decomposition of nutrients into an affordable form for germination. The main enzymes in grain are diastase (dissolves starch and sugar), lipase (fat), a group of proteolytic enzymes that break down proteins and oxidative enzymes - peroxidase, etc. Cereal grain vitamins contain a vitamin complex: carotene as a source of vitamin A, then in small amounts B1, B2, E, K, and PP. Vitamins are mostly found in the germ (Glamočlija et al., 2015; 2017).

As this wheat species originates from the arid areas of Central Asia, khorasan wheat shows greater tolerance to abiotic stress caused by drought, which indicates possible directions of regionalization in Serbia (Alkhammas, 2017). The production year has the great impact on wheat productivity (Malešević et al., 2005; Popović, 2010; Filipović and Ugrenović, 2010; Ugrenović, 2013; Jankovic et al., 2015; Lakić et al., 2015; Ugrenović et al., 2018; Terzić et al., 2019; Rajičić et al., 2020). This study aimed to determine the effect of t supplementary nutrition on the productivity and chemical composition of wheat grains.

MATERIAL AND METHODS

The subject of the study was the population of khorasan white awn wheat, optional type. The influence of supplementary mineral nutrition of plants on the chemical composition of wheat grains was investigated. Experimental field micro-experiments were performed in 2015/2016, in the Danube region, on chernozem-type soil, by the method of a random block system in four replications. The size of the experimental plots was 6 m^2. The experiments were performed in three dietary variants: control (without mineral nutrients) and NPK 72:45:45 and NPK 99:45:45. Mineral nutrients NPK 15:15:15 were introduced with pre-sowing preparation in the amount of 300 kg ha^{-1}, and CAN in February by fertilizing crops. In the second variant, 100 kg ha^{-1} CAN was used, and in the third variant 200 kg ha^{-1}. After the harvest, the seeds were taken for chemical

analysis (Glamočlija et al., 2017; Alkhammas, 2017). The chemical composition of the grain was tested in the Laboratory for Pharmacognosy of the Faculty of Pharmacy in Belgrade. The analyzes determined: the amount of starch, total proteins, cellulose, total lipids, mineral salts, and the amount of water (dry matter). Starch, oils, and mineral salts were determined using the method developed by Kaluđerski and Filipović (1998). To determine the amount of starch polarimeter a CARL ZEISS 24/60 Hz with a specific rotation angle of 181.3° was used. The content of total proteins was determined by the Kjedahl method and converted by a factor of 6.25 (ISO 20483:2006). Total cellulose was determined by the Veender method using the Fibertec 2010 system. All examined parameters were analyzed using the statistical package Statsoft 12, ANOVA, and are presented in Tables and Figures.

Meteorological conditions

The examined production year 2015/2016 was favorable for the production of khorasan wheat. The total amount of precipitation as well as the average temperature during the vegetation period (total 638 mm and 11.7°C (in vegetative period 510 mm and 8.7°C,) respectively - were lower than the multi-year average of these parameters (total year 691.0 mm and 2.4°C, respectively (in vegetative period 518 mm and 7.9°C)), Figure 1.

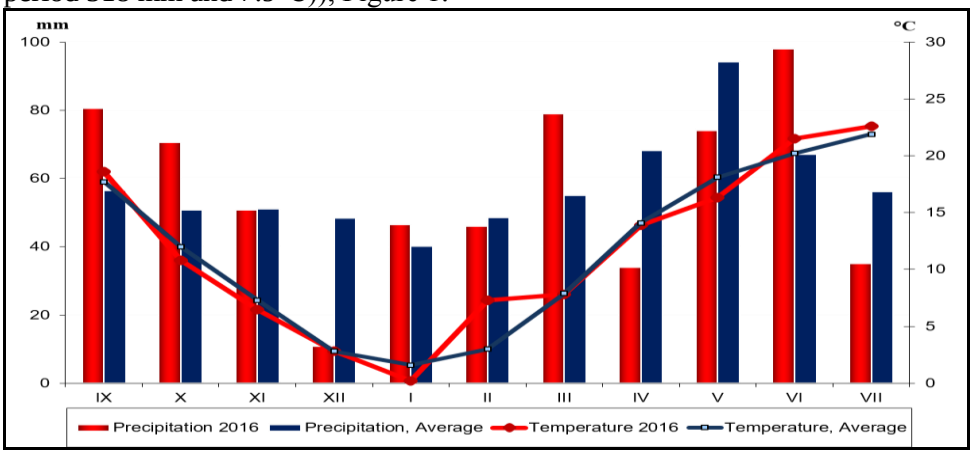

Figure 1. Temperature and precipitation of vegetation period of khorasan wheat

An temperature conditions with mild winters, moderately warm springs, and warm summer months were favorable for Khorasan wheat production.

RESULTS AND DISCUSSION

Productive characteristics of plant mass, number of spikelets, spike mass, grain mass, and harvest index are shown in Table 1. All examined factors varied and were statistically significantly higher for productive traits and qualitative traits (protein and cellulose content), although values of other tested quality

parameters, in variants with supplementary nutrition, were higher compared to the control variant, but the difference was not significant (Tables 1-3).

The great influence of nitrogen on the productivity of khorasan wheat plants was confirmed in the researches of Glamočlija et al. (2015 and 2017) and Alkhammas (2017). The results of these researches indicated that the use of a smaller amount of nitrogen significantly increased the yield parameters. The highest values were achieved with a larger amount of nitrogen. The mass of plants varied from 3.2-3.8 g, the number of spikelets from 21-26, the mass of spikelets from 1.5-1.7 g, grain yield per plant from 1.1-1.81 g, and the harvest index from 35-49%, (Table 1, Figures 2 and 3).

Table 1. Yield parameters of khorasan wheat

Parameter / **NPK**	**Plant mass (g)**	**Number of spikelets**	**Spikelet mass (g)**	**Grain mass (g)**	**Harvest index (%)**
I – Control	3.20 ± 0.15	21± 0.10	1.5± 0.13	1.10± 0.11	35± 2.15
II	3.50 ± 0.11	25± 0.12	1.6± 0.15	1.70± 0.12	49± 2.11
III	3.80 ± 0,12	26± 0.13	1.7± 0.12	1.81± 0.15	48± 1.16
Average	3.5 ± 0.27	24± 0.25	1.61 ± 0.14	1.53± 0.35	44± 6.92

Parameter		**Plant mass***	**No. of spikelets***	**Spikelet mass***	**Grain mass***	**Harvest index***
LSD	0.5	0.212	0.210	0.209	0.199	3.461
	0.1	0.303	0.302	0.303	0.303	5.24

Mineral nutrients affected the total amount of dry matter and the chemical composition of khorasan wheat grains, increasing its nutritional value (Table 2).

Table 2. Chemical composition of khorasan wheat grain (%)

NPK*	**DM**	**PC**	**OC**	**Starch**	**Cellulose**	**NFE**	**MS**
I	89.74±1.09	14.94±0.43	22.4±0.20	61.49±1,25	38.23±0.25	18.34±0.25	14.82±0.05
II	86.87±0.13	15.17±0.05	23.1±0.62	67.82±1,15	39.10±0.11	18.35±0.55	14.73±0.35
III	86.90±0.15	15.20±0.20	22.7±0.15	67.90±1,10	38.71±0.12	18.31±0.25	15.76±1.71
Average	**87.84**±0.10	**15.14**±0.43	**22.7**±0.15	**65.74**±1,95	**38.68**±0.45	**18.34**±0.43	**15.10**±1.11

*NPP-plant nutrition, DM-dry matter, PC-protein content, OC-oil content, NFE-nitrogen free extractives, MS-mineral salts

Parameter		**DM**	**PC***	**OC**	**Starch**	**Cellulose***	**NFE**	**MS**
LSD	0.5	1.267	0.347	0.765	40.655	0.033	0.079	2.010
	0.1	1.920	0.525	1.159	61.589	0.499	0.119	3.044

In the control variant, kamut grains had 89.74% of dry matter (10.26% of water), while in the variants with NPP mineral nutrients they had less dry matter - 86.90% (13.10% of water), which indicates the extension of the vegetation period of plants in conditions of enhanced mineral nutrition (Alkhammas, 2017). Similar

results were obtained by Janković et al. (2011) studying the influence of nitrogen on the yield and grain quality of barley, i.e. wheat and oats by Rakić et al. (2012a and 2012b). Mineral nutrients, that primarily increased quantity of nitrogen, had a positive effect on protein synthesis in plants.

Average protein content was 15.14%. Khorasan wheat grains had 14.94% of total proteins in control, and 15.20% in variants with NPK nutrition, that is 4.46% more (Table 2, Figure 4). Plant nutrition did not have a greater influence on the oil synthesis in the grains, so the difference between the control variant (22.4%) and the variant with NPK mineral nutrients (22.7%) was 15.68%. This difference is the result of bouncing and better-poured grains in plants in the NPK mineral nutrition system.

A statistically significant difference between variants (control, and plant nutrition) was in the amount of cellulose. The amount of digestible cellulose in kamut grains grown without supplementary nutrition (38.23%) was statistically significantly lower, by 0.9%, compared to the variants with NPK mineral nutrients (39,10%), (Table 2, Figure 4).

Khorasan wheat grains in control had less NFE than in supplementary plant nutrition variants. Also, the content of mineral salts in the grains of control plants was lower compared to the variants of supplementary nutrition, which is a consequence of less photosynthetic activity and faster maturation of plants.

(a) (b)

Picture 1. Khorasan wheat plants in the experimental field (a), khorasan wheat grains (b)

The identification of those biological processes that act as markers in tropical wheat is essential for its cultivation across new agricultural frontiers, the promotion of new farming practices, and the attainment of higher yields (Ferreira et al., 2019). The ecological exam is necessary to clarify the true adaptability and suitability of winter wheat variety for regional conditions (Nazarenko et al., 2019).

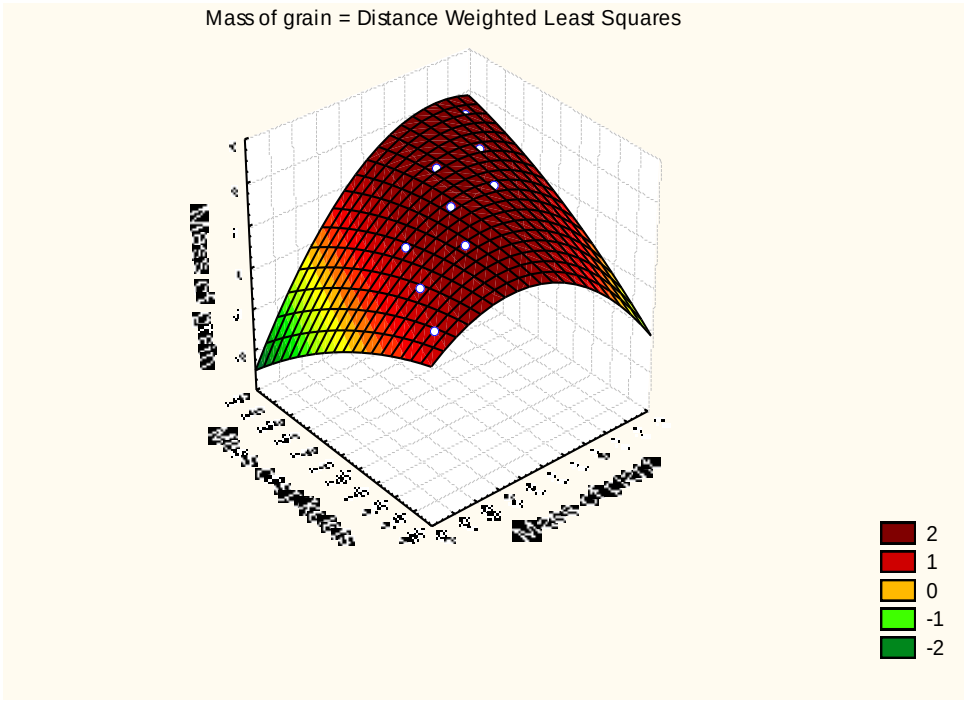

Figure 2. 3D Surface plot for the mass of grain, the mass of spikelets and the mass of plant

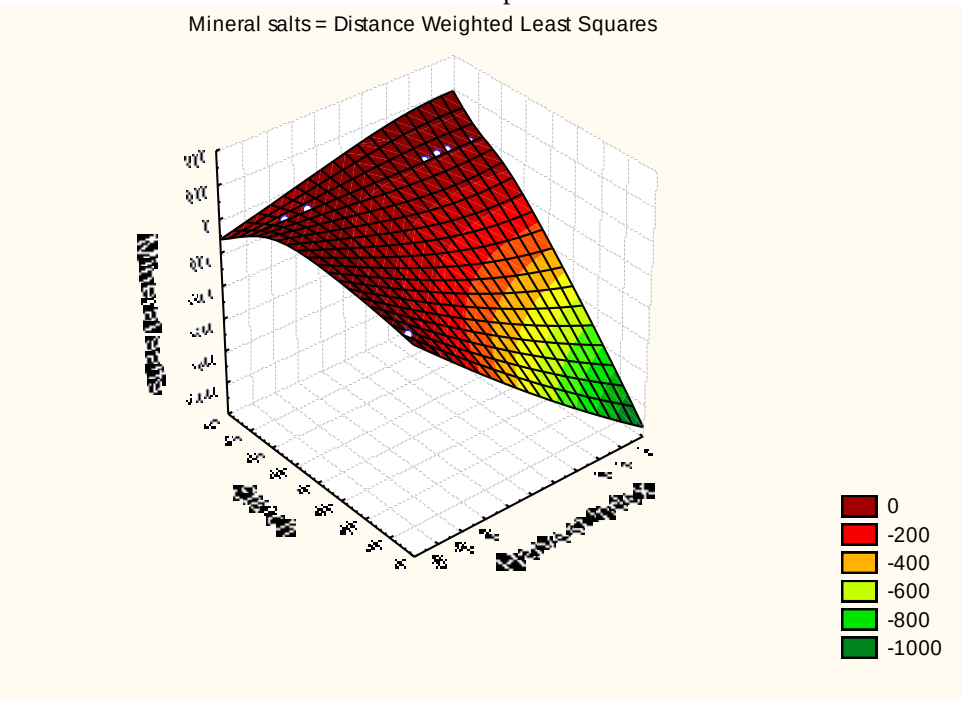

Figure 3. 3D Surface plot for harvest index, starch and mineral salts

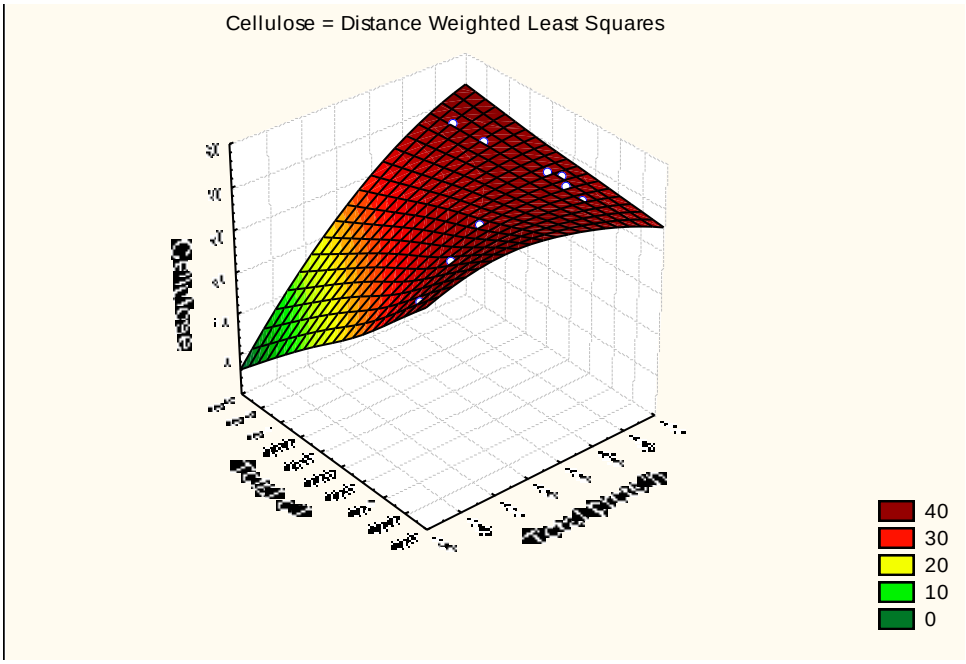

Figure 4. 3D Surface plot for cellulose, total oil, and total protein content

Analysis of the total amounts of polyphenols and antioxidant values of khorasan wheat grain showed that its grain has a higher values use (Table 3).

In the tested khorasan wheat grain samples, the NPP variants showed that the content of total polyphenols was in the range of 10.50-10.85 mg GA/g, which is on average higher than in the control samples (9.44 mg GA/g). The total antioxidant capacity of the tested extracts (FRAP value) was in the range of 39.48-45.61 μmol Fe^{2+}/g of extract. The FRAP values of the samples in the variant with NPP were higher than the FRAP values of the control (Table 3).

Table 3. Total polyphenols and antioxidant activity of khorasan wheat grains

Variant	Total polyphenols[a] (mg GA/g)	FRAP[b] (μmol Fe^{2+}/g)	DPPH[c] (mg/ml)
I Control	9.44±0.60	39.48±4.12	1.78±0.77
II Variant*	10.50±0.69	44.12±2.57	1.81±0.92
III Variant	10.85±0.75	45.61±6.45	1.76±0.14
Average	10.16 ±0.73	42.87 ±3.20	1.76 ±0.05

NPP*-supplement nutrition, [a]total polyphenols (expressed as mg of gallic acid-GA per gram of dry ethanol extract); [b]total antioxidant capacity in μmol Fe^{2+} per gram of dry extract, [c]concentration of investigated extract that neutralizes 50% of free DPPH radicals (SC50) in mg/ml

The reactivity of potential antioxidants to the stable DPPH radical was measured by the DPPH test, and the concentrations of the extracts that neutralized 50% of the DPPH radicals were in the range of 1.76-1.81 mg/ml. These results

are consistent with some previous results of anti-DPPH activity studies of different wheat cultivars (SC50 0.6–7.1 mg/ml) (Yu et al., 2002; Alkhammas, 2017). As with the FRAP test results, there was no significant difference in anti-DPPH activity between the tested samples in the DPPH test. The shown antioxidant activity depended on the content, but also on the type of polyphenolic compounds in the tested samples.

The growing interest in ancient grains appears in recent literature, because of their nutritional and health properties, and especially on KAMUT® khorasan wheat. Any khorasan wheat sold under the KAMUT® brand must follow several quality specifications related both to the nutritional characteristics and growing conditions (i.e. the grain must be grown organically) (Bondet et al., 1997; Quinn, 1999). Some studies demonstrated that KAMUT® khorasan wheat has the high carotenoids content (Abdel-Aal et al., 2007) and a unique nutraceutical value for its peculiar content in bioactive phytochemicals (Dinelli et al., 2009).

Furthermore, KAMUT® khorasan wheat is rich in selenium. It has been demonstrated that selenium content in KAMUT® khorasan bread was ten-fold higher than in modern durum bread (Piergiovanni et al., 2009; Gianotti et al. 2011; Benedetti et al., 2012). Selenium acts in the active site of several enzymes involved in cellular protection from oxidative damage, such as glutathione peroxidase (Xia et al., 2007) and other selenoproteins.

CONCLUSIONS

The quality, i.e. the nutritional value of khorasan wheat grains is the most important characteristic of this real grain, which distinguishes it from the group of other species of the genus *Triticum*. Analyses of the amounts of the most important nutrients: total proteins, starch, sugar, digestible cellulose, and mineral salts have shown that this wheat grown in the agroecological conditions of the Danube region has a great nutritional value. The amount of these substances was mostly influenced by the enhanced mineral nutrition of plants, so that their increased values justified additional investments in NPP mineral nutrients, especially the protein and cellulose content.

Analysis of the total amounts of polyphenols and antioxidant values of khorasan wheat grains showed that its grain has a higher use value compared to grains of other species of the genus *Triticum*. The obtained results of the content of total polyphenols, FRAP, and DPPH test showed higher values in the samples with applied NPK fertilizers compared to the control.

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grains genetic resources towards increased sustainability of grain-value chain and improved farmers' livelihoods in Serbia and Bulgaria – GRAINEFIT.

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DEMOGRAPHIC REVITALIZATION OF MONTENEGRIN RURAL AREAS THROUGH THE SMART VILLAGE CONCEPT

SUMMARY

Rural areas are becoming an increasingly important alternative of living and working choice over cities. Limiting factors for the development of rural areas are depopulation and deagrarization. The industrialization that followed World War II conditioned migratory movements from rural to urban areas.

The paper analyses the application of the concept of "smart villages" in rural areas of Montenegro, with special emphasis on demographic revitalization. Based on the analysis of demographic trends in Montenegro, in addition to participation of urban and rural population in the 1955-2020 period, it implies that previous models and strategies did not contribute to a significant demographic revitalization of rural areas.

Application of the "smart village" concept in the region and beyond shows that traditional models of rural community development must be complemented by digital technologies and innovations.

The modern development of Montenegrin rural areas indicates modern communication in preforming the business, along with the use of internet marketing, which, in addition to easier access to information, will contribute to the improvement of the quality of life in general.

Key words: smart village, depopulation, rural areas, digitalization

INTRODUCTION

Smart village is a new concept in the field of EU policy-making. The Cork Declaration of 2016, entitled Better Life in Rural Areas, presented the CAP (Common Agricultural Policy of the EU) plan for more efficient rural development. Motives for launching new models of rural community development, applying the "smart villages" concept, are specifically the responses to depopulation and demographic aging of villages, using modern technologies, improving e-literacy and e-skills of rural residents, aiming at improving the quality of their life and work (Ristić and Bosković, 2020).

European rural areas face the challenges of unemployment and depopulation. Differences exist and depend on the position of the rural area in

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relation to the main urban areas (peri-urban areas, areas well connected to the main urban centres or remote rural periphery), in addition to the geographical position within the European Union. European policies dealing with the promotion of smart villages are as follows: Common Agricultural Policy, Cohesion Policy, Horizon 2020, Digital Union.

Rural areas are large, vast, covering up to 80-85% of national territories. According to the OECD methodology, a community is considered rural if the population density is less than 150 inhabitants per km^2, while a community is considered urban if the population density is higher than 150 inhabitants per km^2. According to the OECD methodology, the entire territory of Montenegro could be considered rural (Ministry of Agriculture and Rural Development, 2019). The contemporary challenge is to connect dimensions of agricultural and rural changes and contribute to socially, ecologically and economically balanced development (Knickel and Rentin, 2000). Multipurpose agriculture could become one of the key economic branches, but it is to be based on knowledge, modern technologies, in order to ensure safe and innovative products while respecting responsible resource management (Ristić, 2016). Sustainable agriculture is gaining in importance and implies the rational use of natural resources (Stojanović, 2016). The essence of sustainable development of rural communities is an improvement of the quality of life of the population and protection of the environment (Pugliese, 2001).

In recent times, it is clear that urban development is increasingly intensive at the global, European and national level. An important factor for the further development of society is development of rural areas. Trends characteristic for rural areas, both globally and in Montenegro, are the following: negative demographic trends, declining number of agricultural holdings, economically conditioned depopulation of rural communities and growing challenges in terms of access to public services, employment opportunities and overcoming the existential issues.

Rural areas incline to closer urban centres, establishing connections related to the satisfaction of basic services: finance, education, health services, trade, entertainment, culture, etc. In this process, "smart villages" are perceived as modern communities, acting proactively on relevant contemporary challenges, in order to find new opportunities for survival and development (Ristić and Bošković, 2020).

The key factors for the development of smart and competitive villages are considered to be: good connections - broadband connection of rural areas, in order to overcome their isolation and increase accessibility; use of modern services; digital market access; farm modernization; diversification of the rural economy and sustainable use of rural resources (Thorpe et all, 2016).

The conceptual boundaries of the smart village are defined on the following assumptions (Visvizi and Lytras, 2018):

a) a village is an ecosystem of limited size,
b) a smart village is conceptually different from the construct "rural area",
c) focus in smart village research is on the rural population,
d) smart village has a emphasised pragmatic orientation in an effort to diagnose the problem and offer a solution

Matter of socio-economic sustainability in the context of a smart village is crucial, as it defines the perspectives and survival of the rural community (Visvizi and Lytras, 2018). Research (Carmen et all, 2018) indicates that farms face major challenges due to lack of sustainability. Smart villages do not refer just to agriculture. In the context of the European Union, the "smart villages" concept refers to rural areas that rely on existing forces and assets, as well as to the development of new opportunities. In smart villages, traditional and new networks and services have been improved through digital, telecommunications technologies, innovation and better use of knowledge, all for the benefit of residents and their businesses (Zavratnik et all, 2018). The smart village enables its residents to use modern technological and social aspects and achievements, while the infrastructure is developed in accordance with the principles of sustainable development (Zavratnik et all, 2018). Carefully formed smart villages will provide a basic framework for the local population to improve their economic and social status, living conditions and thus strengthen their community that will be more flexible to the challenges of the outside world (Srivatsa, 2015).

The area of Montenegro is a basis for the development of the entire population of the country, strengthening the essential purposeful use of spatial potentials, while preserving the diversity of landscapes and biodiversity. Regional specifics are the basis for achieving the local, regional and international identity of Montenegro and its constituent areas. For the creation of smart villages, one should rely on good practices of other countries, yet should take into consideration local and regional differences, yet nevertheless it is vital to improve the quality of life of the rural population (Bocinell et all, 2015). The vitality and development of rural areas largely depends on the availability of public services and infrastructure in rural communities and cities in rural regions. Urban-rural connections are especially pronounced in the context of road and other infrastructure planning. Good road connections and internet access are prerequisites for the technological progress of rural communities (Matković, 2017).

All previous efforts and models of rural development have not significantly contributed to the demographic renewal of rural areas. Human capital is a foundation of the development of rural areas that are of great importance in facing socio-economic, demographic, environmental and other challenges. Montenegro is the first country in the region to adopt a Smart Specialization Strategy for the 2019-2024 period. Agriculture represents a significant place within the Strategy, aimed of strengthening the value chain of organic production and the development of new agricultural products. The set goals provide the

possibility of a new concept of development of "smart villages" through the application of information and communication technology to improve life in them. The new development model can contribute to improving the demographic picture and creating conditions for the return of young people (Smart Specialization Strategy of Montenegro, 2019).

In order for rural areas in Montenegro to be further developed in modern conditions, it is necessary to use human capital effectively, which refers to the level of formal and non-formal education, quality of labour force, development of entrepreneurial spirit and culture, modern way of thinking, etc. Thus, conditions are created for endogenous resources to be used for solving socio-demographic, economic and environmental issues in a modern and innovative way.

The aim of this paper is to point out the importance of revitalization of rural areas, with special emphasis on demographic revitalization, and through the introduction of the concept of "smart villages".

MATERIAL AND METHOD

The paper analyses dynamics and forms of depopulation of rural areas in Montenegro. The official data of the Statistical Office of Montenegro (Monstat) were used in the preparation of the paper, as well as scientific and professional papers that dealt with this issue. Data referring from the Agriculture Census in Montenegro and the Population Census in the period 1948-2011 were used for the analysis. Statistical tables, line and surface graphs were used in order to display the data.

The relative numbers of the structure show the participation of the urban and rural population in Europe and Montenegro in the 1955-2020 period. Dynamic statistical analysis, more precisely, the method of calculating base indices, was also applied. The SMART method[2] was applied to formulate development goals within the concept of "smart village". Methods of description, comparison, analysis and synthesis were used in the paper

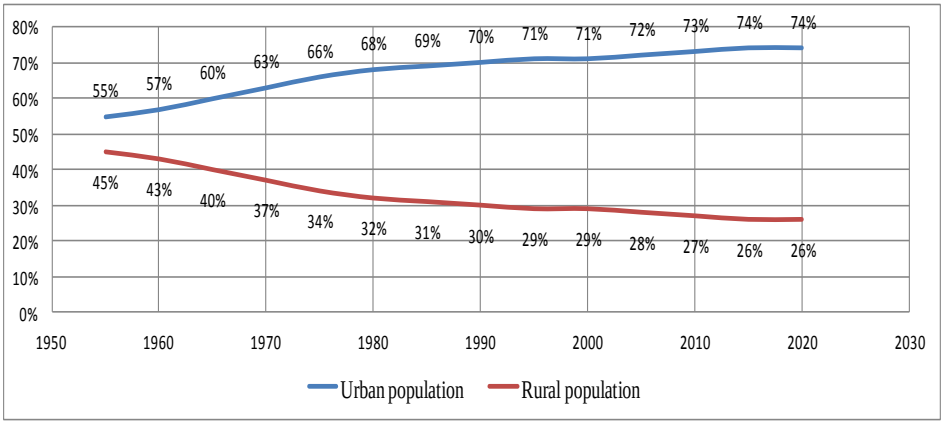
RESULTS AND DISCUSSION

Revitalization of rural areas is one of the key factors in the process of Montenegro's accession to the European Union. Rural areas in Europe are undergoing radical change. In this process, "smart villages" are perceived as modern communities, which act proactively on modern challenges, in order to find opportunities for survival and development (Ristić and Bošković, 2020).

The dynamics of the participation of urban and rural population in Europe in the 1955-2020 period is shown in Chart 1.

[2] The acronym SMART consists of the English words: Specific, Measurable, Achievable, Real, and Timely.

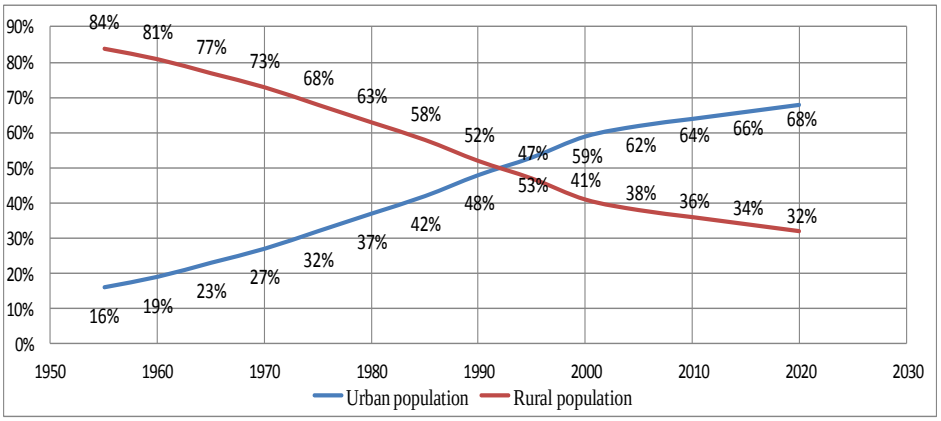
Chart 1. The dynamics of the participation of urban and rural population in Europe in the 1955-2020 period



Source: https://www.worldometers.info/demographics/demographics-of-europe

The dynamics of the participation of urban and rural population in Montenegro in the 1955-2020 period is shown in the Chart 2.

Chart 2. The dynamics of the participation of urban and rural population in Montenegro in the 1955-2020 period



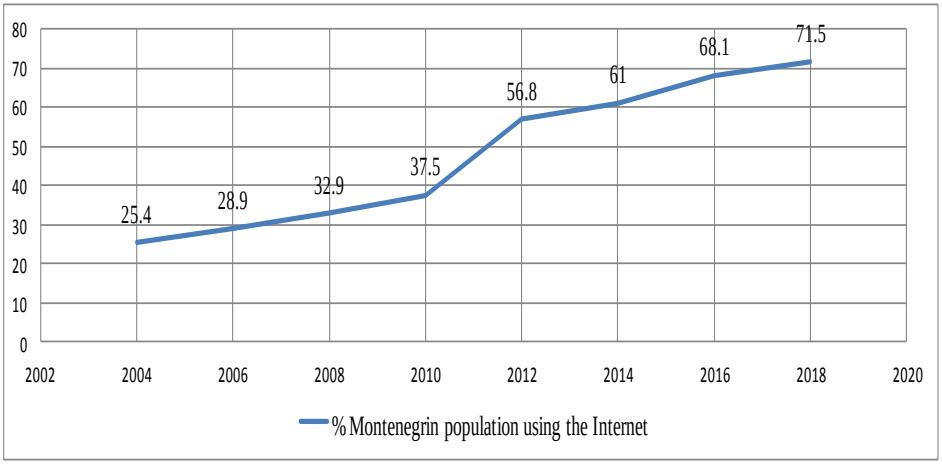
Source: https://www.worldometers.info/demographics/demographics-of-europe

In the 1955-2020 period, there is a tendency to reduce the share of the rural population in the total population, both in Europe and in Montenegro. Complex and intertwined processes of industrialization and urbanization, as well as deagrarization and deruralization have resulted in profound changes (Lukić, 2012). In most EU member states, rural regions have a higher percentage of the elderly population and are exposed to a higher degree of poverty compared to urban areas (European Commission, 2018). The presented demographic situation accelerated the degradation of the local rural world, reduced the standard of living

and the culture of housing. Agricultural land is neglected (Štambuk, 2002). Rural development cannot be based yet only on agriculture. Montenegrin small-scale agriculture survived the Second World War and everyone who had the opportunity left it. The result of leaving the village is an aging agricultural population. Montenegrin urban society developed much faster than rural, believing in its "perfection". The rural area was neglected, therefore schools and clinics were easily closed, there was no rural cultural activity, etc. That is reason why the revitalization of the village today is not just a return to agriculture, but represents the creation of a new social context that relies on the population. Human resources are the main driver of economic development because labour productivity depends on them (Zjalić, 2009, Rakić, 2006). Living conditions in rural areas are very important for the return of people. It is not the same to live in a village, which is about 30 minutes away from the city, and in a remote rural area, which has been cut off by snow for several months. UNDP researches in Montenegro show that the pattern of employment in villages is similar to that in nearby cities, while remote rural areas are much more dependent on agriculture, forestry and etc. (UNDP, 2013). The difficult access of the rural population to modern educational and health institutions is a significant cause of migration in the rural-urban relationship (Ristić and Bošković, 2020). Educational capital is a constitutive element of human capital and, therefore, one of the basic factors of intellectual capital (Koković, 2009, Petty and Guthrie, 2000). About half of EU farmers have secondary education and farmers do not use education enough to modernize their businesses. Regarding household access to the Internet, data for 2018 show that the lowest percentage is in rural areas (85%), in smaller cities and suburban areas 89%, and in large cities 91% (Ristić and Bošković, 2020).

According to the World Bank for 2019, the number of Internet users in Montenegro has been increasing from year to year Statistical Office

Chart 3. Percentage number of Internet users in Montenegro, 2004-2018.



Source: World bank, 2019

When it comes to the territorial representation of the Internet in households, it is the lowest in the northern region of 64.8%, while in the south it is 79.2%. Internet access in non-urban settlements is 62.80%, while in urban settlements access is achieved by about 80% of the population in 2019 (Table 1). (Statistical Office, 2019).

Table 1. Internet access at homes, (%)

Type of settlement	2019	2018
City	80.00	76.30
Other	62.80	60.90
Montenegro	74.30	72.20

Source: Statistical Office, Public Release No. 188

The average Internet use in Montenegro by age and sex in (%) in the period August-October 2019 is shown in Table 2.

Table 2. Average Internet use in Montenegro by age and sex in (%), in the period August-October 2019

Internet use	Age						Sex		Total
	16-24	25-34	35-44	45-54	55-65	65-74	M	F	
Every day or almost every day	99.6	96.3	88.6	79.6	77.9	72.8	87.4	88.5	87.9
At least once a week	0.4	3.7	10.3	17.3	21.0	20.1	11.7	9.3	10.7
Less than once a week	0.0	0.0	1,1	3.1	1.1	7.1	0.9	2.2	1.4

Source: Statistical Office, Public Release No. 188

Referring to the data from Table 2, it can be seen that the persons who stated that they used the Internet in the period August-October 2019, mostly did so every day or almost every day. The percentage of Internet use is almost every day higher for women and amounts to 88.5%, while for men it is 87.4%.

Surveys of the Statistical Office in 2019, regarding the purchase or order of goods or services over the Internet for private purposes, showed that about 69% of respondents did not buy and order goods over the Internet, while 31% of respondents bought and ordered goods over the Internet. The type of goods or services that respondents most often ordered were as follows:

Table 3. Type of goods or services that respondents most often ordered online in 2019 in Montenegro, %

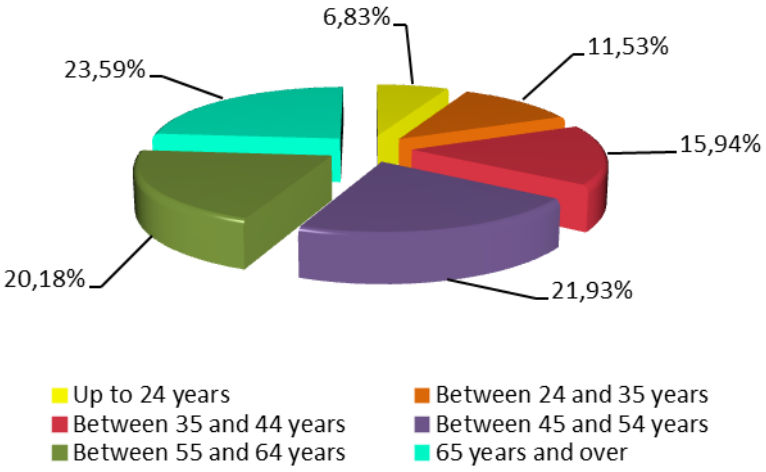
Types of goods or services	Share in order
Clothing, sports products	73.5
Toys	17.2
Pharmaceutical products	11.0
Movies and more	10.8
Video games and other merchandise	5.5

Source: Statistical Office, Public Release No. 188

The data show that the ordering of agricultural and food products has not yet come to life, although a survey was conducted in Montenegro regarding the online promotion of agricultural products (Žarić et all, 2017). There is significant room for improving Internet food sales, namely to promote the "smart village" concept. Advances in information and communication technology in the context of rural communities represent an opportunity for villages in the 21st century to become places for quality and comfortable living. The path to the implementation of the "smart village" concept is not easy, primarily because it is necessary to solve the problem of depopulation and the outflow of young people from rural areas to urban centres.

Depopulation and unfavourable age structure of employees in agriculture in Montenegro are a significant limiting factor in the development of the concept of "smart village". Montenegrin agriculture is characterized by an aging rural population and significantly lower average level of education. More than 44% of the population is older than 55, 65% older than 45. More than half (55.3%) of employees in agriculture graduated from high school, and only 9.1% graduated from high school or college (Agriculture Census 2010, Structure of agricultural holdings).

Chart 4. Age structure of the labour force on family farms in Montenegro



Source: Monstat, 2010

The unfavourable age structure on family farms is a consequence of a number of economic and social factors. Education is a necessary factor in the modernization of rural communities. The majority of the rural population does not have the basic e-skills necessary for success in a society in which digitalization is an important factor in the development and competitiveness of "smart villages" (Ožegović, 2019).

Table 4 shows the percentage of the population aged 15 and over according to education in Montenegro in the 1981-2011 period.

Table 4. Percentage of participation of the population aged 15 and over by education and sex in Montenegro, 1981-2011

		Total	Without education	Elementary school	High School	Higher School	University	No answer
1981	Total	100	13,86	51,36	28,34	2,89	3,26	0,26
	Male	100	6,66	47,9	36,42	3,91	4,76	0,28
	Female	100	20,76	54,63	20,60	1,92	1,83	0,25
1991	Total	100	8,86	45,82	34,97	3,82	5,03	1,48
	Male	100	4,12	41,82	41,02	4,77	6,63	1,65
	Female	100	13,47	49,69	29,12	2,89	3,49	1,31
2003	Total	100	4,30	32,59	48,44	5,03	7,51	2,10
	Male	100	2,09	27,29	53,32	5,93	8,83	2,51
	Female	100	6,38	37,59	43,83	0,41	6,62	1,73
2011	Total	100	2,3	28,16	51,90	5,20	11,92	0,50
	Male	100	0,88	23,33	57,00	6,15	12,12	0,55
	Female	100	3,56	32,80	47,10	4,30	11,73	0,50

Source: Despotović, A.(2016).

The analysis based on the base index (1981 = base) shows a declining trend in the number of inhabitants without school and only with primary school, and there is a trend of increasing the number of inhabitants with secondary and higher education. The largest number of residents has completed high school. According to the 2011 census, the number of inhabitants with secondary school increased by 116% compared to the base year 1981 **(Despotović, 2016).** According to the same author, in the period 1981-2011. The percentage of women without school and with (un)completed primary school ranges from 75.39% according to the 1981 Census to 36.4% according to the 2011 Census.

Demographic changes occurred in the 1965-2011 period influenced the further development of family farms in Montenegro and by region. The importance of agricultural farmers emanate from the fact that almost all production takes place on them (Despotović et all, 2016). In Croatian agricultural policy, family farms also take the main place (Štambuk, 2002).

Based on the aforementioned characteristics of rural areas and the changes that have taken place in the last fifty years, it is not possible to talk about the rapid implementation of the concept of "smart villages". In that direction, it is necessary to recognize the opportunities brought by digitalization, new business models, precision agriculture, bio-economy, etc. as soon as possible.

Modern development of Montenegrin rural areas implies modern communication in business, with the use of internet marketing. This development requires a quality way of financing, increasing the competitiveness of agricultural products, as well as better road connections with urban communities. Total food consumption decreases with the improvement of per capita income levels in urban communities, but with the improvement of the living standard of the population, food consumption tends to be based on better quality products (Jovanović, 2016). The market of organic products in Montenegro is gaining in

importance, with special emphasis on the development of tourist consumption of agricultural products in rural areas of Montenegro (Jovanović et all, 2017).

In some EU countries (Germany, Hungary, Slovenia) pilot projects of good practice of "smart villages" have been implemented. Thus, for example in Slovenia, a series of courses on digital technology were held, thus encouraging young people to stay in the countryside. The pilot project resulted in the digitization of six regional centres, in addition to 35 smaller cities (Ristić and Bošković, 2020). The "smart villages" concept is being successfully implemented in Croatia as an EU member, by encouraging the digitalization of villages.

Based on the experiences of the countries of the region, information and communication technologies are the basis for smart growth and development. In order to harmonize with the Digital Agenda in Europe and the Strategy for the Digital Single Market, Montenegro has adopted a Strategy for the Development of the Information Society until 2020, which defines the strategic directions for the development of the information society (Ministry of Finance, 2017). All social segments should be transformed based on new technologies, with special emphasis on rural areas. The use of information and communication technologies in rural areas would save time and money, as well as easier access to information, which is important for life, private and business, and would contribute to improving the quality of life.

Taking into account the current situation of Montenegrin rural communities, the "smart village" concept should be directed in several directions:

- –Applying digitalization to put agricultural production in the broader context of rural policy through horizontal networking in the local area;
- –Inclusion of vulnerable groups, especially rural women in the process of modernization of life in rural communities,
- –Solving the problems of elderly people left to live alone in villages;
- –Improve mobility, especially of the elderly through improved public transport and taxi services;
- –Innovations in the field of health services, education and financing;
- –Application of digital technologies and the Internet in connecting food producers and consumers;
- –Work intensively on the preservation of Montenegrin tradition and cultural heritage;
- –Encouraging the development of rural entrepreneurship through the diversification of activities on the farm;
- –Improving the environment for the development of agritourism, old crafts, etc.

Previous analysis of the situation in Montenegrin rural communities is a starting point for the application of SMART methods for creating development goals within the concept of "smart village".

Source:https://www.google.com/search?q=SMART+(Specific,+Measurable,+Attainable,+Realistic,+Time+bound

Table 5. SMART analysis of rural community development goals within the concept of "smart village"

S	**Specific**	Digitization, innovation, networking, green econom
M	**Measurable**	Measurement of achieved economic results: effectiveness, efficiency, economy, productivity, profitability
A	**Attainable**	The goals are achievable with permanent education of the population, transfer of knowledge in the field of IT, financial support of the state and local governments
R	**Realistic**	Adapt the concept of "smart village" to the real conditions of rural communities and the real state of agricultural production
T	**Time bound**	Make progress in the development of rural communities in certain timeframes, through socio-economic indicators

Source: Author based on Ristić, Jakšić and Trlaković, 2019; Ristić and Barbarić, 2019

Picture 1. Application of digital technology in agriculture

Source: Žulj M. (2019): Digital Transformation of Agricultural Value Chain_EN-mm

Based on the SMART analysis, it can be seen that the concept of developing a "smart village" in the Montenegrin rural community is achievable with permanent education of the population, knowledge transfer, financial support from the state and local governments. The results of the achieved goals should be visible throughout the food value chain.

CONCLUSION

After the Second World War, Montenegro was an area where migratory movements in the north-south direction were very apparent. This has led to a change in the structure of the population, primarily in age and education. Such changes had a negative impact on the development of agricultural production. Labour shortages continue to be a significant problem in the process of agricultural and rural community development, as human resources are a major driver of economic development on which labour productivity depends. Previous models of rural development have not contributed to the demographic revitalization of rural areas.

The analysis in this paper showed that the “smart village” concept may play a key role in the process of demographic revitalization of rural communities.

The application of the concept of “smart village” in the region, primarily in Slovenia and Croatia, shows that traditional models of rural community development must be complemented by digital technologies and innovations.

The level of efficiency of the application of the mentioned concept in Montenegro will significantly depend on the extent to which the creators, first, of the agrarian policy will recognize its importance. The concept of "smart village" should be adapted to the real conditions of rural communities and the real state of agricultural production. This requires the engagement of the wider community, through the financing of projects for the application of information and communication technologies, followed by the readiness of the population to master new knowledge and skills in using it.

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ATTITUDE AND BEHAVIOUR OF BOSNIAN HOUSEHOLDS TOWARDS FOOD WASTE

SUMMARY

In order to determine the amount and value of food waste, a regional survey was carried out, among others, in the Bosnia and Herzegovina (BiH), at the beginning of 2016. A number of 581 respondents participated in on-line survey and their answers were processed using descriptive statistics and dual-non-parametric test. The results of the research were presented according to logical units: socio-economic characteristics of households; general habits when buying food; attitude toward food products shelf life; attitude toward food waste and factors of rational use of food. Most households in BiH prepare meals at home, and do not often eat in a restaurant or buy ready-made food. Food is mostly purchased in supermarkets and hypermarkets and about half of the respondents discard less than 0.5 kg of food weekly, the value of which is less than 5 euros. The above and other results suggest that food waste in BiH is still not a big problem, which is a consequence of the tradition and way of life, but also of low living standard.

Keywords: Bosnia and Herzegovina, food consumption, food waste, nutrition.

INTRODUCTION

Imbalance in food production and consumption results in starvation in the world, because some of the world's population does not produce enough food, or there is not enough money for purchasing food. On the other hand, there is an inconsistency between purchasing and consumption of food of the most powerful

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consumers, which causes some food waste. Numerous researches have been carried trying to determine the quantities of produced and necessary food.

Insufficient quantities and uneven distribution of food are directly related to the occurrence of hunger, malnutrition, undernutrition, undernourishment. According to the World Food Programme (WFP, 2009), almost 1 billion people struggle to find their next meal, while a joint study of FAO, IFAD and WFP (2015) pointed out that 795 million people are undernourished, mostly in Asia and Africa. Alexander et al. (2013) associated food waste with the failure to use potential edible items to address human hunger. Hence, food losses directly reduce the amount of food used to feed the world's population. According to Gustavsson et al. (2011), food waste at consumer level in industrialized countries is almost as high as the total net production in Sub-Saharan Africa. Lipinski et al. (2013) indicated that most food losses occurred in the final consumption stage in the regions of North America and Oceania (61%) and Europe (52%). This indicates that with the increase in wealth and living standard, food losses due to its non-use and throw-off at household level also increase. Food is lost at various stages of its production (pre, during and post-harvest), processing and consumption (Gustavsson et al. 2011; HLPE 2014). Parfitt et al. (2010) investigated the losses of agricultural products in post-harvest, food processing and retail phases, referring to various research and sources. According to secondary data sources they referred that food waste has recently ranged from 5.4 to 25% in UK, from 12.7% to 25% in USA, 15% in Australia, 26-27% in South Korea and 8-11%in the Netherlands. Jörissen et al. (2015) conducted a research of food losses in which they commented their results referring to more secondary sources about quantity of household food waste per week in different European countries. Aschemann-Witzel et al. (2015) analyzed, in general, causes for consumer-related food waste. In addition to the impact of food waste on food security and nutrition, certain researchers also dealt with the assessment of the impact of these losses on the environment (e.g. Venkat, 2011) as well as the reduction of the household budget available for other needs (Herath and Felfel, 2015). A number of institutions or researchers have estimated food waste during the supply chain. Stenmarck et al. (2016) studied European food waste and within it the household waste found out that at the level of EU-28 in 2012 there were 47 million tons, or 92 kg per person of food waste (where households participate in food waste with 53%). In developed countries, food waste is the subject of statistical monitoring. For example, in Sweden, estimated amount of household food waste increased by 9 kg/person, in two-year period (2010/2012), with estimated unavoidable food waste of (35%) (SEPA, 2012). In Slovenia, almost 14% of all food was wasted before it reached consumer plates (Žitnik and Vidic, 2016). Marangon et al. (2014) had similar study in North-Eastern Italy and Milutinović et al. (2013) in Serbia. It is very important to understand the wastage of food at the household level. The Committee for Economic and Commercial Cooperation of the Organization of Islamic Cooperation (COMCEC, 2017) identified three points of household food waste: between coming into home and

preparation, between preparation and serving and after serving. Capone et al. (2016) noted that food loss and waste prevention and reduction would allow meeting the food needs of about one billion undernourished people.

In line with already conducted researches about food waste, another regional survey has been performed whose results have been published so far for Morocco (Abouabdillah et al., 2015), Egypt (Elmenofi et al., 2015), Lebanon (Chrabel et al., 2016), Turkey (Yildirim et al., 2016), Algeria (Ali Arous et al., 2017), Montenegro (Berjan et al., 2019), and North Macedonia (Bogevska et al., 2020).

In BiH, there was almost no direct research on the topic of food waste, but indirectly some data can be found. Clausen and Pretz (2013) just mentioned food waste in their paper. Statistics authorities record the estimated amount of municipal waste, but do not classify it according to the waste type, so that data on food waste cannot be found there. Both entities in BiH have waste management strategies that treat organic waste as part of municipal waste. In one of two BiH entities, in Republic of Srpska, it is estimated that 0.76 kg of municipal waste is generated daily, out of which organic waste accounts for 34.2% (MSPCE RS 2016).

MATERIAL AND METHODS

The conducted research is a contribution to better understanding of food environment in BiH. HLPE (2017) defines food environment as "physical, economic, political and socio-cultural context in which consumers engages with the food system to make their decisions about acquiring, preparing and consuming food". Since no published results of previous research for the territory of BiH have been found, this paper has a pioneering significance and is the basis for the continuation of similar researches. The aim of the paper was to determine the habits and attitudes of food consumers in BiH in terms of food waste, identify the quantities, types and values of wasted food, as well as the reasons and factors that affect it and those that could reduce this type of food loss. Personal attitudes were collected by the survey method. A database was formed from the collected responses that was further processed applying certain statistical methods.

The survey was carried out on-line at the beginning of 2016, using the Survio software (www.survio.com). The questionnaire was pre-tested in pilot stage. Questions were available in Serbian. Potential stakeholders were invited to participate in survey via email and Facebook. On-line questionnaire consisted of 26 questions into five groups: (1) socio-economic characteristics of households; (2) place, value and habits when buying food; (3) way of storing/preparing food and attitude towards the shelf life of food; (4) quantity, value, types and reasons for food waste; (5) concern regarding food waste. Measurement of response was according to the Likert scale with answers in the range 1-3 or 1-5. All questions were closed type questions, with a number of typified, descriptive responses in the form of statements. On some question multiple responses were allowed. The questionnaire was opened by 1,458 respondents and within 36 days 581

respondents answered completely (the response rate was 40%). Two questionnaires were not suitable for processing, so the sample of responses over which further processing was carried out consisted of 581 questionnaires. All respondents were from the territory of BiH, so that the obtained results are fully relevant for BiH.

Data was processed using SPSS Statistic Software, Version 20. Descriptive statistics and binary nonparametric tests were used for data processing and interpretation of results. Statistical significance testing was done through X^2 independence test. Statistical significance was considered at two levels as highly statistically significant ($P<0.001$) or statistically significant ($P<0.005$).

RESULTS AND DISCUSSION

Socio-demographic characteristics

The analyzed sample consisted of 581 respondents who fully answered all the questions (Table 1). There were more women than men. Young respondents dominated, and those up to 35 years old accounted for 73% of respondents.

Table 1. Social-demographic characteristic of the surveyed sample (n=581)

Characteristic		Frequency	Percent
Gender	Male	239	41.14
	Female	342	58.86
Age (years)	18-24	195	33.56
	25-34	230	39.59
	35-44	108	18.59
	45-54	29	4.99
	55 and over	19	3.27
Family status	Single person or non-related community	55	9.47
	Living with parents	333	57.31
	Married	193	33.22
Level of education	Secondary school	151	25.99
	Technical qualification	15	2.58
	University degree	343	59.04
	Higher degree (MSc, PhD)	72	12.39
Occupation	Paid work (fulltime, part-time or retired)	287	49.40
	Student	179	30.81
	Unemployed, looking for work or home duties	115	19.79
Number of household members	One	26	4.48
	Two	89	15.32
	Three	164	28.23
	Four	183	31.50
	Five and more	119	20.48

The age of the respondents was the reason why only 1/3 of the respondents were married and the rest of them lived with their parents or independently. Regarding the education structure, those with university degree dominated, and as

for the working status about half of respondents (49%) were employed, although the participation of students was significant (31%). The sample was dominated by multi-member families.

Habits of buying and consuming food

Consumer habits explains the results of research that 37% of respondents never eat outside the family (home) or order ready-made food, and 54% of respondents do it less than twice a week. At the same time, 75% of respondents never use ready-made food for preparing meals at home. The survey showed that a negligible number of consumers bought food directly from farmers (1.5%). Food was mainly bought through trade (61%), with the largest share of supermarkets and 37% of respondents bought food in stores. Most often, food was bought every day (35%) and the frequency of purchase decreases as the number of days increases. When buying food, most respondents bought food without a pre-prepared list (72%), which means that when shopping, they were mostly guided by visual and other impulses. This is confirmed by the answers of 37% of consumers who responded that they paid special attention to discounts when buying food, and another 53% said that they did it occasionally. The amount spent by surveyed households on food per month shows that most of them chose the highest interval on the offered scale (more than 150 euros). With the decrease in monthly food expenditure, the number of households was proportionally reduced. Considering the average salary in BiH in the survey period (838 KM, that is 428.5 euros; AS BiH, 2018), it can be seen that almost half of the households spent more than 35% of the average salary on food.

Attitude towards food shelf life

Surveyed food consumers in BiH are quite rigorous according to the food-labelled deadline "use by" date because 81% of them believed that such food should be eaten by that date or thrown after that date. If it is recommended that food is best used up to a certain date, " best before" date, the percentage of those who thought that after that date it should be discarded was smaller (68%). The answers to both questions indicated that consumers were disciplined and that their habits were largely dependent on the shelf-life of food products designated and declared by producers, which means that producers indirectly had the responsibility for the utilization of processed foods, as well as traders in terms of timely procurement planning and placing food on the market.

Attitude towards uneaten food

Answers regarding food waste indicated a high level of consumer responsibility, as 86.9% of the respondents chose the answer indicating that they were concerned about food waste and tried to avoid it (Table 2). Another 8.8% of respondents were aware of the problem of food waste and they were willing to change their behaviour in the future, so the number of those who were indolent to this phenomenon was quite small (<5%).

Table 2. Consumer profiles regarding food throwing

	Statement	Response (%)
1.	I'm worried about throwing food and trying to avoid it whenever I can	86.92
2.	I do not consider throwing food an important problem	4.30
3.	I've been aware of the problem of throwing food away, but I do not think I will see my behaviour in the future	8.78

The answer to the next question is in correlation with the previously stated concern regarding food waste as 59.5% of the respondents confirmed that they discarded almost no or discarded very small quantity of food. Another 1/4 of them thought it was a reasonable quantity, so the number of those who discard more food than it should be discarded was 15%.

Table 3. Quantity of discard food

	Statement	Response (%)
1.	More than it should	15.49
2.	Reasonable quantity	24.96
3.	Almost nothing	20.14
4.	Very little	39.41

The way of life in BiH influenced the answer to the question "What do you do with uneaten food?" so that about 1/3 (31%) of households throw uneaten food into the waste bin (municipal waste), and even 2/3 (65%) of respondents used that food to feed animals (in addition to pets, such as dogs and cats, also birds). The number of those who made compost or donated food was negligible (<1%).

Table 4. Treatment of united food

	Statement	Response (%)
1.	Throwing in a trash can	31.67
2.	Feed animals	65.06
3.	Other	3.27

The frequency of food waste reflects a certain degree of responsibility, as 10.6% of respondents did not discard food ever, and 53.5% did it less than once a week.

Table 5. Frequency of food waste

	Statement	Response (%)
1.	More than twice a week	7.7
2.	1-2 times a week	28.2
3.	Less than once a week	53.5
4.	Newer	10.5

This was confirmed by the distribution of the answers to the question of how often the main meal was prepared from fresh products, because 30% of the

respondents did it daily, adapting the quantity of food to daily needs. Of course, due to the modern lifestyle, there was a large number of those who prepared the main meal less than twice a week (60%).

Table 6. Frequency of preparing the main meal of fresh ingredients

	Frequency	Response (%)
1.	More than seven times a week	29.95
2.	3-6 times a week	59.55
3.	Less than two times a week	10.50

Therefore, it is not surprising that there was a large number of people who did not eat meal from the previous day more than twice a week (70%).

Table 7. Frequency of eating meals from the previous day

	Frequency	Response (%)
1.	More than seven times a week	2.93
2.	3-6 times a week	14.63
3.	Less than two times a week	82.44

The main reasons for food waste are summarized in Table 8. The most common answers were expiration date (for processed foods), or food leftover after meals. Very frequent reasons were, also, long stay in fridge and deterioration of food organoleptic properties.

Table 8. Main reasons for food waste in households (n=1.391)[1]

	Reason	Frequency	Percentage (%)
1.	Food leftovers	238	41.0
2.	Expired date of food	236	40.6
3.	Food has been in the fridge for a long time	224	38.6
4.	Food does not look good/eatable	176	30.3
5.	Food has unpleasant smell and taste	147	25.3
6.	Food was mouldy	131	22.5
7.	There was an error in planning/buying food	68	11.7
8.	Incorrect food storage	60	10.3
9.	Poor cooking skills	11	1.9
10.	Labels lead to confusion	11	1.9
11.	The package was not of an appropriate size	7	1.2

[1] It was possible to select more than one answer.

The quantity of food waste weekly was generally below 1 kg, and as many as 43.7% responded that they did not have any food waste at all.

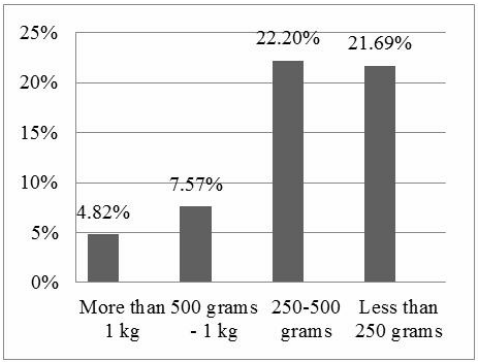

Figure 1. Quantity of food waste weekly

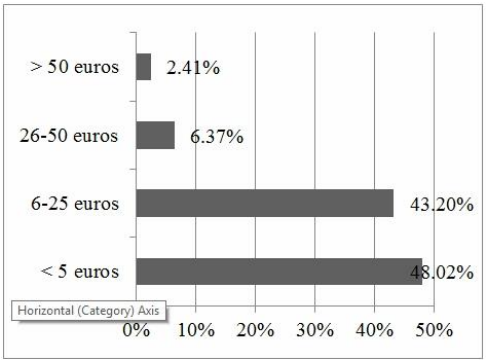

Figure 2. Value of food waste monthly

The value of food waste is relatively small, usually less than 5 euros per month. The relatively small quantity and value of food waste are the consequences of a lifestyle (preparing hot meals in the household and consuming cold foods that do not have to be eaten immediately), the tradition, and a low standard of living that does not allow the scattering of modest income.

Food waste was mostly connected with bread and milk (1/4 of households discarded more than 5% of bakery products, and 15% of respondents discarded more than 5% of dairy products). Food waste regarding fish and meat was very low.

Table 9. Quantity of food waste in a household regarding its type

		Cereals and bakery products	Roots and tubers	Pulses and oilseeds	Fruits	Vegetables	Meat and meat products	Fish and seafood	Milk and dairy products
1.	< 2%	48.4	63.5	70.1	72.4	68.3	75.3	84.7	62.3
2.	3% - 5%	25.7	21.4	20.4	17.8	22.1	14.6	11.2	22.6
3.	6% - 10%	13.7%	10.5	6.5	5.0	4.5	5.3	2.9	9.3
4.	11% - 20%	6.0	2.4	2.1	2.7	2.9	3.9	0.5	2.9
5.	> 20%	6.2	2.2	0.9	2.1	2.2	0.9	0.7	2.9

Factors of rational use of food

After fact that there was not a lot of food waste, it was surprising that the meal from the previous day was not consumed very often (70% less than twice a week). This suggested good planning of preparing meals and adjusting their size to daily needs. Most of the respondents thought that they would have had less food waste if food packages had been more suitable regarding the size, or if they had been better informed about the negative consequences of such actions (Table 10).

Table 10. Reasons that would positively affect the reduction of food waste (n=773)[2]

	Reason	Frequency	Percentage (%)
1.	Better information about the negative impact of food waste on the environment	208	26.91
2.	Better information about the negative impact of food waste on economy	106	13.71
3.	More suitable food packages	217	28.07
4.	More clear food labelling	75	9.70
5.	Some sort of tax on food waste	167	21.60

[2] It was possible to select more than one answer.

X^2 test was used to analyze the association between socio-economic characteristics and behaviour of the consumers. Out of 26 questions, 6 were related to the socio-economic characteristics of the respondents, or their households. In 3 questions, more than one answer was possible, so cross-examination of statistical significance (cross tabulation) for some of the characteristics of the sample could be done in the case of responses to 17 questions.

Table 11. Cross-examination of the impact of consumer characteristics on the relation to purchasing, using of food and food waste

		Gender	Age	Education	Employment	Marital status	Family size
P1	Place of food purchase	0.032	-	0.118	-	0.085	-
P2	Frequency of food purchase	0.183	0.334	0.131	0.157	0.055	0.032
P3	Food expenses per month	0.002*	0.000**	0.003*	0.000**	0.000**	0.208
P4	Use of shopping lists	0.005	0.210	0.768	0.186	0.008	0.004*
P5	Reaction to special offers/discounts	0.001*	0.008	0.695	0.004*	0.253	0.631
P6	Best before (date)	0.601	0.037	0.064	0.043	0.044	0.463
P7	Use by date	0.092	-	-	0.207	0.005	-
P8	Concern regarding food waste	0.015	-	0.365	0.009	0.166	0.786
P9	Quantity of uneaten food/ food waste	0.328	0.087	0.879	0.183	0.183	0.449
P10	Method of discarding uneaten food	0.449	0.048	-	0.008	0.000**	0.000**
P11	Frequency of food waste	0.453	0.469	0.647	0.417	0.728	0.314
P12	Preparation of fresh food meals	0.804	0.378	0.203	0.584	0.001*	0.000**
P13	Eating yesterday's meal	0.083	-	-	0.036	0.005	-
P14	Eating out or eating fast food	0.284	-	-	-	-	-
P15	Eating ready-made food	-	-	-	-	-	-
P16	Quantity of food waste	0.098	0.100	0.817	0.082	0.544	0.196
P17	Value of food waste	0.412	-	-	0.322	0.772	-

Legend: - data not valid; * P<0.05; ** P<0.01

The cross-comparison of the characteristics of the sample and their habits showed that gender had a statistically significant effect on the amount of monthly expenditure on food and the response to special offers. The age of respondents and education had highly statistically significant impact only on the amount of monthly expenditure on food. Employment had a high statistical impact on the amount of food expenditure and a statistically significant impact on reactions to special offers. Marital status had a highly statistically significant effect on the amount of monthly expenditure on food and the treatment of uneaten food. The size of the family had a highly statistically significant impact on the treatment of uneaten food and the preparation of fresh food meals, and statistically significant influence on the use of a preprepared shopping list for food purchases.

Having an insight into the results of the same research in Algeria (Ali Arous et al., 2017), Lebanon (Charbel et al., 2016), Turkey (Yildirim et al., 2016), Morocco (Abouabdillah et al., 2015) and Egypt (Elmenofi et al., 2015), the discussion also provides an overview of the results of the research from BiH in relation to the results from those countries.

Regarding the place of purchase, consumers from BiH usually buy in super and hypermarkets, and very rarely directly from the producers. This is a consequence of the expansion of supermarket chains in BiH in recent years, which has led to a rapid decline in the number of small shops. Also, opportunities for direct purchasing is low (e.g. green markets, are small, as their number is constantly decreasing). Consumers from BiH most buy frequently food because even 65% of them buy food every day or every second day. Also, after the Moroccans, they do not frequently use the pre-arranged list, and do not respond to special offers and discounts. If compare the expenditure for food, consumers in BiH spend less compared to consumers from most other countries. The reason could be their small purchasing power, although other countries do not have much higher average income. Consumers in BiH have expressed the highest level of concern over food waste. Consumers in BiH spend more time eating food from fresh ingredients at home; they rarely eat yesterday's meal the next day, and very rarely eat out. Regarding the quantity of food waste, consumers in BiH are among the most conservative, and the reasons for food waste are roughly the same as in other analysed countries. For some of the factors it was not possible to make a comparison, because the results of data processing were not presented in the same way.

CONCLUSION

Given that there have been no previous surveys on household food waste in BiH, this study makes one of the first contributions in this regard. It showed that consumers in BiH spent a lot of money on food, having in mind their total income. Probably because of this, they were quite rational in terms of planning, ways of preparing, and the frequency and quantity of food waste. Moreover, the amount and value of food waste were quite small. The majority of consumers had a good perception of food shelf life, although there was a fairly high percentage

of those who believed that food could be eaten even after the expiration of recommended shelf life. A high number of consumers was worried about food waste, and they are ready for certain steps that could reduce food waste generation in Bosnian households. This pointed to the need for better consumer awareness on how to store, prepare and alternatively use food. The conducted research had a pioneer character in the territory of BiH and its results could certainly be utilised to plan specific actions in the direction of reducing food waste. It also sets up a baseline for research activities on household food wastage in BiH that would be undertaken in the future.

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EFFECTIVENESS OF SOME NATIVE DIATOMACEOUS EARTH AGAINST MAIZE WEEVIL, *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE), UNDER CONTROLLED CONDITIONS

SUMMARY

The Maize weevil, *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae), is one of important pests of storage maize in Turkey. Laboratory experiments were carried out to investigate the effect of two local diatomaceous earth (Aydın and Ankara) and Silicosec® (Biofa, Germany) against *S. zeamais* adults at 25 and 30°C and 50-65% relative humidity. Maize grains (cultivar Decalp 6664) were treated with three doses of DEs (1000, 1500 and 2000 ppm) and untreated seeds were regarded as control. The dead adult counts were made 2, 3, 5, 7, 14 and 21 days after DEs. The progeny of *S. zeamais* was determined separately for each treatment after 60 days from end of the experiment. The highest mortality (100%) of the maize weevil was observed with 2000 ppm Ankara DE on 14th day after application at 25 °C, which was followed by Aydın and SilicoSec® DEs (99.0 and 90.1%). The insecticidal activity of native DEs increased with increasing application dose, exposure time and temperature. The highest insecticidal activity at 30 °C was determined for Ankara and Aydın DEs with 2000 ppm on 14th day (100%), followed by SilicoSec® (97.9%). All doses of Ankara DE had the highest insecticidal activity than rest of the DEs examined. New adult emergence (F1 offspring) was recorded in all DEs treatments; however, emergence was very low than control treatment. It is concluded that the native DEs (Ankara and Aydın) are very effective and promising against *S. zeamais* adults and might be used for its successful control in storage maize.

Keywords: Diatomaceous earth, *Sitophilus zeamais*, efficacy, storage maize, pest, mortality

INTRODUCTION

Storage grain pests, mainly beetles and moths, such as *Sitophilus* spp. (Coleoptera: Curculionidae), *Tribolium* spp. (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Coleoptera: Bostrychidae), *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Oryzaephilus surinamensis* Linnaeus

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(Coleoptera: Silvanidae) are especially responsible for weight, germination of stored grains and cause losses up to 100% in Turkey (Özar and Yücel, 1982; Özer et al., 1989; Işıkber et al., 2005).

The maize weevil, *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae), is one of important storage pest on maize in Turkey (Yıldırım et al., 2012; Karaağaç and Konuş, 2015) as well as other countries (Trone, 1994; Rees, 2004; Ojo and Omoleye, 2016). The pest also cause problem on small grains for example wheat, barley, sorghum, millet and causes significant quantitative and qualitative damage to storage grains and grains can weight loss up to 90% for not treated storage maize (Giga et al. 1991; Yıldırım, 2012; Ojo and Omoleye, 2016). Insecticides are mainly used for its control in Turkey (Karaağaç and Konuş, 2015) as well as biological control and botanical insecticides among others (Akob and Ewete, 2007). Besides, several alternatives control methods (natural products such as, palm oil, tobacco extract, leaf essential oils, seed extracts and inert dusts) have been studied against (Foua-Bi, 1993; Korunic, 1998; Owusu, 2001; Doumbia et al., 2014) including *S. zeamais*.

Dusts, such as diatomaceous earth, are known insecticidal or repellant activity products and effectively controls most species of insect pests of storage products (Ebeling, 1971; Sousa et al., 2013). Diatomaceous earth affects larvae and adults, stick to insect's body parts as they move on their surface or inside the treated grain mass (Kavallieratos et al., 2007; Rojht et al., 2010; Shams et al., 2011; Sousa et al., 2013). When dust contacts with insects, it causes the removal of the layer of wax from insect cuticle, bringing about their death by dessication (Rojht et al., 2010). Besides, this product is extremely stable and has low toxic effect on mammalian, little potential for insect resistance as well as a good residual effect (Eldridge,1964; Ebeling,1971; Korunic,1998). Turkey has a huge and good quality diatomaceous earth reserve (125 million tons) and most of them geological deposits occur in some regions (especially Center Anatolia, Aegean region) of Turkey (Çetin and Taş, 2012). The regional deposits of DEs in Turkey, their low cost, easy availability and use warrant their usage as grain protectants. Although abundant and high-quality DEs reserves exist in Turkey, these have not extensively been evaluated for their potential utility in controlling storage products pest.

Many studies have been conducted in Turkey and other countries to determine the insecticidal, biological and repellent activity of native and trade DEs against storage product pests (Korunic, 1998; Masiiwa, 2004; Ferizli and Beris, 2005; Athanassiou et al., 2004; Sousa et al., 2013; Doumbia et al, 2014; Ertürk and Emekçi, 2014; Wakil et al., 2015; Alkan et al., 2019; Şen et al., 2019; Kılıç and Mutlu, 2020). Whereas, biological efficacy and dose studies of various DEs have been conducted such as on wheat, rice and corn related to *S. oryzae* and *S. granarius* species in Turkey, there is no literature available related to efficacy of local DEs for *S. zeamais* on storage corn kernels. In this study, some native diatomaceous earth from Aydın and Ankara provinces were evaluated for efficacy against *S. zeamais* in the laboratory-controlled conditions in 2020.

MATERIAL AND METHODS

Diatomaceous Earth (DE) formulations

Three diatomaceous earth (DEs) (two natives, i.e., Ankara, Aydın and imported, i.e., SilicoSec®) were used in the study. The DE formulations were given in Table 1.

Table 1. Diatomaceous earth formulations used in the study.

DE*	SiO_2 (%)	Al_2O_3	Fe_2O_3	CaO	Na_2O	Particle diameter size (µm)
Ankara	92.8	4.2	1.5	0.3	-	8-12µ
Aydın	94.2	4.6	1.6	0.3	-	8-12µ
SilicoSec®**	92.0	3	1	-	1	8-12µ

*DE: Diatomaceous earth

**A sample of dry formulation of Silicosec® was obtained from Biofa GmbH, Germany.

Test insects

Sitophilus zeamais adults were obtained from cultures maintained in the Entomology Laboratory of Plant Protection Central Research Institute Ankara, Turkey. The adults were reared in sterile 2-L transparent plastic jars on maize grains in incubators maintained at about 25±1°C and 65±5% relative humidity and 16:8 h (L: D) in Insectarium of Plant Protection Department, Agriculture Faculty, Harran University. Maize grains were kept in the deep freezer at -18 °C at least 7 days to prevent contamination. The jars were not filled to the brim for allowing free air circulation and respiration. Approximately, 250-300 mixed-sex adult individuals were introduced in the jars. The adults on the maize grains were vacuumed after 24 hours for obtaining 1-day-old new adults later. The newly emergence adults were observed at ~35-40 days after. Emerged adults were collected with an aspirator and used in experiments when they were 1 to 7 days old.

Laboratory bioassay

Bioassay studies were conducted according to randomized parcel design with four replicates at two temperatures (25 and 30±1ºC), 50-65% RH. Three doses of local (Ankara and Aydın) and SilicoSec® DE formulation (1000, 1500 and 2000 ppm (mg DE/kg maize grain) were used. Untreated maize grains were considered as the control. For each dose, 100 g maize grains were used in 1-liter volume plastic jars. The jars were sealed and shaken manually for 1 min to distribute DE equally. Thirty *S. zeamais* adults were introduced in each jar and the insects were kept in controlled condition insectarium rooms at 25 and 30±1°C. The numbers of living and dead adults were recorded after 2, 3, 5, 7, 14 and 21 days respectively.

Effect of diatomaceous earth on F1 progeny

The dead and living adult insects were removed 21 days after the initiation of laboratory bioassay. The same jars (infested maize grains) were kept at 25 and 30±1°C and 65±5% relative humidity condition to allow oviposited eggs to hatch

and develop. Then newly emerged adults were counted. Inhibition rate was calculated according to the formula below.

$$IR = (Cn - Tn) / Cn * 100$$

IR% : Inhibition rate
Cn : Number of newly emerged insects in the untreated (control) jar
Tn : Number of newly emerged insects in the treated jar.

Statistical Analysis

All data were converted into percent mortality and subjected to arcsine scale followed by correction of cumulative mortality percentage for the corresponding control mortality (Abbott, 1925). Analysis of variance was conducted to assess the effect of concentration, time of exposure and their interaction with the insect mortalities. The differences among treatments were analyzed by means of Tukey multiple comparison tests ($p<0.01$). All statistical analyses were conducted with Jump (Version 7) package program.

RESULTS

The mortality varied within DEs and their doses. The highest mortality was recorded at higher DEs dose (2000 ppm/100g maize grains) at 25 and 30°C, while minimum mortality was obtained with lower dose. The lowest mortality after 2nd day was 31.12, 36.09 and 13.33% with 2000 ppm dose of Ankara, Aydın and SilicoSec® DEs, respectively at 25 °C (Table 2).

Table 2. Efficacy of different doses of three diatomaceous earth against *Sitophilus zeamais* adults at 25 °C

		Mortality (%)					
DE	**Treatments**	**2. DAT**	**3. DAT**	**5. DAT**	**7. DAT**	**14. DAT**	**21. DAT**
Ankara	Control	0.83 a	0.83 a	0.83 a	0.83 a	5.83 a	13.33 a
Ankara	1000 ppm	3.33 b	10.08 a	29.45 b	38.99 b	48.24 b	75.70 b
Ankara	1500 ppm	26.87 c	62.93 b	84.08 c	89.08 c	95.33 c	100.00 b
Ankara	2000 ppm	31.12 c	65.54 b	91.66 c	96.60 c	100.00 c	100.00 b
		Mortality (%)					
Aydın	Control	0.83 a	0.83 a	0.83 a	0.83 a	4.16 a	10.00 a
Aydın	1000 ppm	5.86 ab	17.58 a	30.20 b	39.51 b	54.89 b	67.40 b
Aydın	1500 ppm	15.91 b	46.89 b	79.02 c	87.47 c	93.98 c	99.16 c
Aydın	2000 ppm	36.09 c	71.35 b	90.69 c	93.25 c	99.03 c	100.00 c
		Mortality (%)					
SlicoSec	Control	0.00 a	0.00 a	0.00 a	0.00 a	6.66 a	10.83 a
SlicoSec	1000 ppm	2.50 a	3.33 a	10.83 b	15.00 b	38.28 b	84.12 b
SlicoSec	1500 ppm	10.00 b	27.50 b	51.66 b	60.00 c	90.13 c	99.10 c
SlicoSec	2000 ppm	13.33 b	28.33 b	60.00 b	77.50 d	91.06 c	100.00 c

*Different letters in the same column indicate statistically different from each other ($P<0.05$)
DAT: Day after treatment

Increasing of exposure time resulted increased *S. zeamais* adult mortality. The 1000 ppm dose of all DEs was insufficient, mortality increased significantly 5 days after treatment (DAT) with 1500 ppm and reached 84.08% with Ankara DE. The efficacy of Aydın DE was similar to Ankara DE (Table 2). After 7th day, 2000 ppm dose of Ankara and Aydın DEs exhibited high mortality (96.60 and 93.25%), while mortality with the same dose of SilicoSec® (77.50%) was lower. Insecticidal activity was >90% with 1000 ppm; however, 100% mortality was recorded with 2000 ppm dose of Ankara DE 14 DAT. However, SilicoSec® did not reach 100% mortality with all concentrations. The mortality rate at 21 DAT was 100% with 1000 ppm doses of all DEs. The mortality with 1000, 1500 and 2000 ppm doses of all DEs at 30 °C was substantially greater than control at 3 DAT. A considerable increase in mortality was observed with increasing duration in control treatment but remained the lowest (Table 2).

The mortality percentages of *S. zeamais* adults at 2, 3, 5, 7, 14 and 21-DAT at different concentrations of Ankara, Aydın and SilicoSec® DEs at 30 °C are shown in Table 3.

Table 3. Efficacy of different doses of three diatomaceous earth against *Sitophilus zeamais* adults at 30 °C

		Mortality (%)					
DE	**Treatments**	**2. DAT**	**3. DAT**	**5. DAT**	**7. DAT**	**14. DAT**	**21. DAT**
Ankara	Control	1.66 a*	2.50 a	3.33 a	4.16 a	16.66 a	29.99 a
Ankara	1000 ppm	28.79 b	66.77 b	88.81 b	89.44 b	98.81 b	100.00 b
Ankara	1500 ppm	48.94 bc	77.15 b	89.85 b	93.96 b	99.13 b	100.00 b
Ankara	2000 ppm	53.45 c	87.26 b	97.38 b	97.38 b	100.00 b	100.00 b
		Mortality (%)					
Aydın	Control	0.00 a	4.99 a	8.33 a	8.33 a	17.50 a	30.83 a
Aydın	1000 ppm	35.83 b	61.36 b	78.90 b	84.42 b	92.76 b	98.33 b
Aydın	1500 ppm	69.16 c	83.24 c	96.39 bc	97.29 bc	98.10 b	100.00 b
Aydın	2000 ppm	70.83 c	86.75 c	97.32 c	98.21 c	100.00 b	100.00 b
		Mortality (%)					
SlicoSec	Control	0.00 a	3.33 a	6.66 a	6.66 a	23.33 a	40.00 a
SlicoSec	1000 ppm	4.16 ab	9.48 a	33.83 b	51.11 b	80.40 b	83.61 b
SlicoSec	1500 ppm	4.16 ab	23.07 b	42.19 bc	63.26 bc	85.54 b	98.68 b
SlicoSec	2000 ppm	12.50 b	31.13 b	58.67 c	78.44 c	97.95 b	98.75 b

*Different letters in the same column indicate statistically different from each other ($P<0.05$)
DAT: Day after treatment

Exposure time of insects to different DEs concentrations had significant effects ($P<0.01$) on mortality of *S. zeamais* adults at 30 °C (Table 3). Increasing DEs doses and exposure time resulted in increased mortality. The mortality after 72 h was 87.26 and 86.75% for Ankara and Aydın DEs, respectively with 2000 ppm dose. SilicoSec® caused very low mortality 31.13% with same exposure time.

The highest mortality (97.3%) was recorded with 2000 ppm concentration of Ankara and Aydın DEs on 5th and 7th DAT, and reached 100% on 14th DAT, while SilicoSec® caused 58.67 and 78.44% mortality on 5 and 7th DAT, respectively. The highest mortality (98.75%) for SilicoSec® was noted 21 DAT and did not reach 100% with any application doses. Mortality in the untreated control remained constant after the insect exposure to untreated maize grains.

Different DEs and application doses showed different suppression effects on the progeny production of *S. zeamais*. After the long storage period (60 days), F1 adult emergence was recorded from all DEs and their all concentrations. The highest number of insects emerged from Aydın, Ankara and SilicoSec® were 30, 26.5 and 15.5 adults respectively in the lower concentration treatment (1000 ppm) at 25 °C. The F1 adult numbers were decreased with increasing applications doses (1500 and 2000 ppm), which were 1, 2.5 and 7.8 adults for Ankara, Aydın and SilicoSec®, respectively at 2000 ppm (Figure 1).

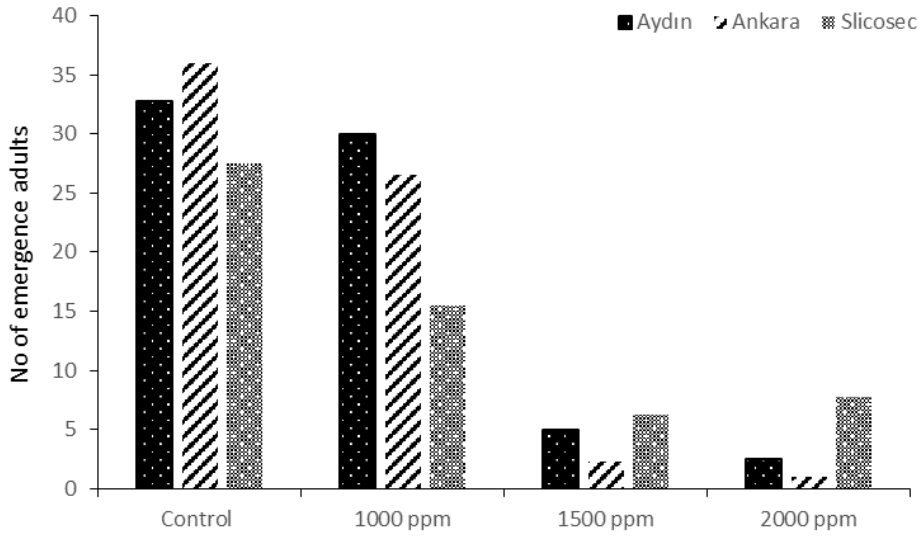

Figure 1. Progeny assessment after 60 days of *Sitophilus zeamais* exposure to the various DE at 25 °C

Unlike the data shown in Figure 1 of 25 °C, F1 adult emergence was very low with all doses of tested DEs with no differences between all DEs at 30 °C. The number of insects emerged, at the end of the exposure period of 60 days, reduced with the increasing DE doses (Figure 2).

The highest dose (2000 ppm) of Ankara and Aydın DEs was very effective in terms of suppression of F1 production (Figure 2). The number of insects emerged from grains treated with DEs at 1500 and 2000 ppm at 30 °C was substantially lesser than the grains in the control

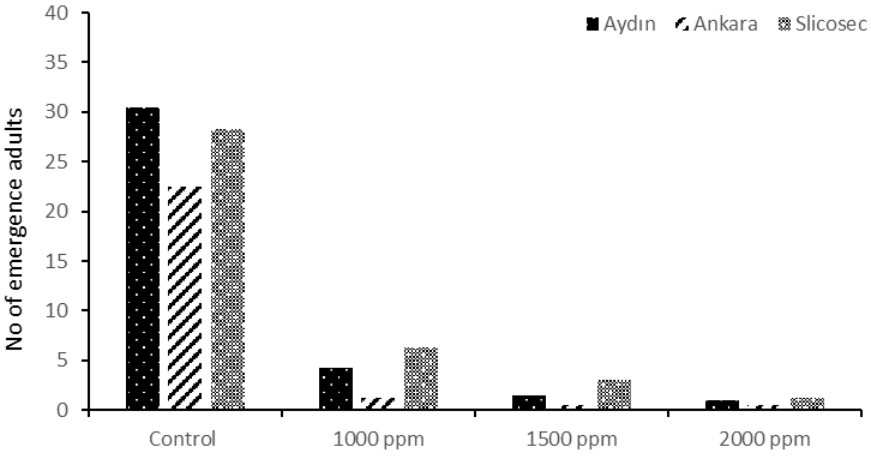

Figure 2. Progeny assessment after 60 days of *Sitophilus zeamais* exposure to the various DE at 30 °C

DISCUSSION

The purpose of this study was to investigate the effect of two native diatomaceous earth (DEs) on mortality of *S. zeamais* adults under controlled conditions. The results of the study indicated that the efficacy of all DEs was considerably influenced by doses, time of exposure, temperature and type of DE formulations. Similar results have been reported by previous researchers (Korunic, 1998; Arthur, 2001; Arnaud et al., 2005; Vayias et al., 2006; Shams et al., 2011; Alkan et al, 2019; Şen et al., 2019; Kılıç and Mutlu, 2020). These studies indicated increased mortality of storage product pests exposed to inert dusts with increasing exposure time. Exposure time of two-day of DEs was not enough to obtain significant mortality with tested DEs in the current study. However, the mortality rate was increased at three DEs, as increased day of exposure after 5^{th} day and reached higher mortality rate on 14^{th} day at 25 and 30°C in the study. Similar result reported by Arthur (2001) who stated that mortality of *Oryzaephilus surinamensis* L. generally increased as exposure interval and temperature increased. Extended exposure time may be needed to increase mortality in adults, because more dust particles would be trapped by their bodies, losing more water and causing death by desiccation (Fields and Korunic, 2000; Arthur, 2001; Rigaux et al., 2001; Shams et al.2011).

The effectiveness of DEs increased with higher doses and temperature. This effect shows that the mortality of insects is dependent on the conditions in which the grains are exposed to DEs (Sousa et al., 2013). The particles and size of DEs have abrasive peculiarities but more importantly the ability to absorb lipid molecules from the cuticle of the insects and subsequently, in water loss, dehydration and death (Quarles and Winn, 1996; Korunic, 1998; Subramanyam

and Roelsi, 2000). Altough the particles size of all DEs is very close, SilicoSec® had moderate effect on *S. zeamais* adults. The results indicated that DEs from different locations vary in their efficacy against the maize weevil. This has also been explained by Korunic (1997) and Golob (1997) that DEs from different geological locations have different efficacies and DEs from marine areas are the most common but less efficacious.

The mortality was considerably increased with increasing temperature and was higher at 30ºC than 25ºC. The earlier studies on the influence of temperature on the efficacy of some local and traded DEs against storage pest (*S. oryzae*, *T. confusum* and *R. dominica*) indicated that increasing temperature resulted in increasing insecticidal efficiency against *S. oryzae* adults (Fields and Korunic, 2000; Arthur, 2002; Athanassiou et al., 2005; Vassilakos et al., 2006; Rojht et al., 2010, Şen et al., 2019). The results of the current study agree with Vassilakos et al. (2006) who reported that insecticidal efficacy of SilisoSec® against *S. oryzae* increased with increasing temperature. However, increased temperature would also increase feeding and therefore moisture replacement through the food and production of metabolic water. The synthesis of cuticular waxes may be faster at higher temperatures because of temperature effects on the biochemical pathways (Turnbull and Harris, 1986; Şen et al., 2019). High temperatures stimulate the movement of insects within the grain mass, allowing them greater contact with DEs. In addition, water loss is likely to be increased at higher temperatures (Arthur, 2001; Fields and Korunic, 2000).

The total mortality was influenced by DE types under both temperatures. The local DEs, Ankara and Aydın, showed >90% mortality 5 DAT, while SilicoSec® caused ~60% mortality at 25°C. Smilar results were obtained at 30 °C which clearly indicated that the local DEs were effective than SilicoSec® against the maize weevil. The differences between SilicoSec® and local DEs can be explained by the differences in physical, morphological and chemical characters of DEs. Kılıç and Mutlu (2020) found the highest biological activity with local DE Aydın on *Trogoderma granarium* Everts larvae followed by Ankara DE, while SilicoSec® had the lowest activity larvae at 30°C. However, Alkan et al., (2019) reported that the local DE Turco000 caused 100% mortality to *Acanthoscelides obtectus* Say. adults on chickpea at 1000 ppm 4 DAT and can be used as a valuable tool for bean weevil suppression in storage product pest management programs. Besides, complete mortality of *T. confusum* and *S. oryzae* can be achieved at lower concentrations ranging from 500 to 900 ppm local DE formulation (ACN-1) and this DE has potential to be used for control of storage-grain insects reported by Şen et al. 2019.

The study results showed that at least 1500 ppm dose at 30 °C caused >90% mortality 5 DAT. The results of the present study indicated that complete mortality of *S. zeamais* achieved at higher concentrations, ranging from 1500 to 2000 ppm. These results do not agree with Masiiwa (2004) who reported different results with local African DEs on the maize weevil. Insect mortality caused by DEs is attributed to the dehydration provoked by the abrasiveness of the small

particles of this inert dust and by adsorption of oils in the body of the insect, which breaks the layer of wax on the epicuticle, exacerbating the fatal loss of water reported by Subramanyam and Roesli (2000). Therefore, at higher concentrations, the adsorption of wax and abrasiveness caused by the product occurs faster, causing death in a shorter time compared with low concentrations (Shams et al. 2011). In addition, the higher the concentration of DEs can be the more effective, because dust applied to cover containers and grain surface will be greater chances of the insect picking up the dust to cause enough damage (Masiiwa, 2004). Maize grain surface is rough, and adherence is greater than small grain like wheat, millet which are smooth surface have low adherence.

The number of emerged adults in progeny reduced with increasing temperature, reaching ~5% at 1500 and 2000 ppm doses of all DEs at 25 and 30°C. The high mortality was observed for 30°C 5 DAT. These results could be due to the fact that higher temperatures might reduce the rate of oviposition and limit the survival of insects and reduce the number of individuals in the progeny (Vardeman et al., 2006). Alkan et al. (2019) reported that there was no progeny production with local DEs, Turco000, Turco004, Turco020 at 200 ppm concentration at the end of 55-day incubation period on *C. maculatus*. Kılıç and Mutlu (2020) stated that 1000, 1500 and 2000 ppm of local DEs (Aydın and Ankara) applied to wheat resulted low mortalities of *T. granarium* larvae and higher doses did not prevent reproduction.

The results presented in this study suggest that 1500 ppm dose of Aydın DE with high temperatures can be recommended to control the maize weevil with 5 days exposure time.

CONCLUSIONS

The native diatomaceous earths obtained from local sources (Ankara and Aydın) showed high efficacy against the maize weevil under controlled conditions. The study also indicated that temperature had significant effect on the insecticidal efficacy of local DEs against the maize weevil, *S. zeamais*. Based on the results of the bioassays, mortality increased with increasing dose and the exposure time. In conclusion local DEs (Ankara and Aydın) have potential to be used for control of maize weevil.

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ANALYSIS OF THE HAIR TEXTURE OF THE BOSNIAN BROKEN-HAIRED HOUND – BARAK

SUMMARY

Bosnian broken-haired hound called Barak is the only broken-haired breed from the Balkans recognized by FCI, standard Nº155 from 1973. The standard describes the hair of the Barak as long, hard, shaggy, tousled, with a dense undercoat. The basic colours of the hair is wheaten yellow, reddish yellow, earthy gray and dark gray. The colour can be combined in bicolour and tricolour. The standard does not define the length of the hair.

The Austrian Frank B. Laska (1905) conducted the first zootechnical examination on hounds from the territory of Bosnia and Herzegovina and classified them by the type of hair. Since 1905, the population of this breed in Bosnia and Herzegovina has not been the subject of research, its hair accordingly.

The aim of this paper was to collect samples and analize the texture and quality of hair cover of Bosnian broken-haired hound – Barak The study involved 30 dogs, including 22 males and 8 females, from the territory of Bosnia and Herzegovina.

The average length of hair was 7.03 cm for males and 6.67 cm for females, with large variation interval. Based on the results of covercoat hair, it can be concluded that this dog has coarse and hard hair, which completely justifies the very name of this breed (broken-haired). The results from this research can be used for further detailed description of this breed.

Keywords: Barak, hair texture, hound

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INTRODUCTION

According to today`s official systematics accepted in cynology, the Fédération Cynologique Internationale (FCI, 2019) divide dogs into 10 groups, and the group of hound dogs is classified in group 6, which includes 3 sections (section 1 has 3 subsections) with total of 76 dog breeds:

FCI group 6	Scent hounds and related breeds
Section 1	Scent hounds
Section 2	Leash (scent) Hounds
Subsection 1.1	*Large-size Hounds*
Subsection 1.2	*Medium-size Hounds*
Subsection 1.3	*Small-size Hounds*
Section 3	Related breeds

(Fédération Cynologique Internationale – FCI, 2019)

The Austrian Frank B. Laska (1905) conducted the first zootechnical examination on hounds from the territory of today`s Serbia and Bosnia and Herzegovina. The measurements were performed on 1036 hounds, and the results were published in the book Das Waidwerk in Bosnien und der Hercegovina (Laska B.F, 1905). According to the author, the Balkans are the nursery of hounds, that is, the hounds from the Balkans were the basis for creation many breeds of hounds on the West. Laska classified the hounds from these areas by the type of hair into:

1.Straight-haired (short-haired) hounds

2.Long-haired hounds

3.Broken-haired (rough-haired) hounds

For each group of hounds, the author gave detailed description in the form of standards. These are the first standards written for the hounds from the Balkans.

Bosnian broken-haired hound called Barak is the only broken-haired breed from the Balkans recognized by FCI and classified in group 6, section 1, subsection 1.2. The official Standard Nº155 was adopted in 1973, and has not been changed since. The standard describes the hair of the Barak as long, hard, shaggy, tousled, with a dense undercoat. The basic colours of the hair is wheaten yellow, reddish yellow, earthy gray and dark gray. The colour can be combined in bicolour and tricolour. The standard does not define the length of the hair.

Since 1905, the population of this breed in Bosnia and Herzegovina has not been the subject of research, its hair accordingly. So, when it comes to hair cover of the Bosnian broken-haired hound called Barak, there are no references in

available literature that directly defines the texture and quality of the hair (Nikitović, 2020).

Besides, the lack of register of indigeneous breeds of domestic animals, with precise data on the species, locations and number of individuals, makes it imposible to take measures for protection as well as prevention of illegal exports, which endangeres their survival (Nikitović et al, 2015).

The aim of this paper was to collect samples and analize the texture and quality of hair cover of Bosnian broken-haired hound – Barak. We were not able to determine whether the obtained values meets the values defined by standard for this breed, as the standard only descibes the look and the colour of hair, but gives no data on the length and diameter of the hair.

The hair texture of this broken-haired hound will give us information of how this dog adapted to harsh climate conditions of mountain forested regions of Bosnia and Herzegovina. They must have such a coat that allows them to stay out in the field for a long time in various weather conditions (Urošević et al, 2009). This research will also give us the insight of the justification of this breed`s name itself.

MATERIAL AND METHODS

The research involved 30 dogs, including 22 males and 8 females, from the territory of Bosnia and Herzegovina. The analysis of the texture and quality of the hair was conducted using lanimeter manufactured by Reichert-Jung.

The samples were taken by cutting the strands of hair with scissors to the skin, from the left side of dog`s body. Three strands of hair (one from the shoulders, one from the back and one from the croup) were taken from each dog involved in this research. There were more than 300 hairs in each strand of hair.

Three cross-sections with scissors were made on each of the samples. The first cross-section was made at the bottom, where the undercoat and cover hair are located. The second cross-section was made at the middle of the hair, an the third at the top, where the covercoat is located. After the sections were completed, each was placed on a microscope plate, one or two drops of glicerine was added, and covered with another plate. Counting of the hairs in each of the samples, as well as measurements of hair diameter were performed using lanimeter.

In order to obtain results of hair texture, the software *Statistical Package for the Social Sciences (SPSS) for Windows Release 17.0.0* was used to process collected data. Descriptive statistic values were calculated: coefficient of variation (CV), standard deviation (SD), minimum value (MIN), maximum value (MAX) and mean ($\bar{x}$).

RESULTS AND DISCUSSION

According to the exterior characteristics, the closest breed to Bosnian broken-haired hound Barak is Bulgarian long-haired hound Barak, though still not recognized by FCI. For the purpose of defining the exterior parameters of

Bulgarian Barak, a hair analysis was conducted on the sample of 20 male and 20 female individuals (Urošević et al. 2018). The authors state that the average diameter of cover hair was 21.59 μm (micrometer), while the undercoat hair had an average diameter of 12.89 μm. When it comes to separate regions, from where the samples were taken for examination of hair diameter, the results show that the largest diameter of cover hair was on shoulders (22.51 μm), and the smallest diameter was on the croup (21.12 μm). The authors state that a large variation interval was found for this parameter. Thus, the diameter of cover hair on shoulders varied from 5 μm to 70 μm. Variation interval for the cover hair diameter on the back had the range from 4 μm to 66 μm, while on the croup it varied from 3 μm to 66 μm. When it comes to undercoat, this study shows that the average value of undercoat hair diameter is 12.89 μm, with variation interval from 3.00 μm to 34.00 μm. The largest diameter of underoat hair was found on the croup, with an average value of 14.00 μm, with variation interval from 3.00 μm to 30.00 μm. The smallest diameter of undercoat hair was found on the shoulders. The average value was 11.94 μm, with variation interval from 4.00 μm to 34.00 μm. Considering the values of the diameter of the covercoat hair, as well as the undercoat for Bulgarian hound Barak, it is obvious that this breed has coarse hair.

The hair of the Bosnian shepherd dog – Tornjak on the territory of Bosnia and Herzegovina was examined by Salkić et al. (2009). The study involved 42 dogs originated from two locations, Potocani (21 dog) and Imljani (21 dogs). The dogs lived on significantly different altitudes. On the lower altitude location (Potocani), the largest diameter of covercoat hair was found on the shoulders, with an average value of 65.68 μm, and variation interval from 56.03 μm to 72.25 μm. The smallest diameter of covercoat hair was found on the back, with an average value of 55.07 μm. Variation interval ranged from 47.02 μm to 59.46 μm. When it comes to the croup, the covercoat hair averaged at 58.28 μm, with variation interval from 49.72 μm to 62.46 μm. At the same observed location, a fairly homogenized diameter of the undercoat hair was found. On the shoulders, the average value was 25.67 μm, with a minimum of 22.97 μm and a maximum of 27.17 μm. On the back, the average value of the undercoat hair diameter was 25.54 μm, with variation interval from 22.27 μm to 28.53 μm. Speaking of the croup, the average value of the undercoat hair diameter was 25.78 μm, with a minimum value of 24,53 μm, and a maximum value of 27.06 μm.

On the other location (Imljani, mountain Vlasic), the average diameter of covercoat hair on the shoulders of Tornjak was 65.68 μm, with variations from 56.03 μm to 72.25 μm. The hair on the back and on the croup had smaller diameter. The average value of covercoat hair on the back was 55.07 μm, with minimum of 47,02 μm and maximum of 59.46 μm. The covercoat hair on the croup was 58.26 μm on average, with variation interval from 49.72 μm to 62.45 μm. When it comes to the diameter of undercoat hair on the shoulders, the average value was 25.67 μm, with minimum of 22.97 μm and maximum of 27.17 μm. The diameter of undercoat hair on the back averaged at 25.54 μm, with

variation interval from 22.27 µm to 28.53 µm. The average value of the undercoat hair on croup was 25.78 µm, a minimum was 24.53 µm, and a maximum was 27.06 µm.

There are only three dog breeds (Bulgarian long-haired hound - Barak, Bosnian and Hercegovinian-Croatian shepherd dog - Tornjak and Romanian shepherd dog - Mioritic), whose hair texture and quality was analized and described using this method, prior to this research.

The tables show the results for both males and females.

Table 1. Values of length and diameter of hair cover - males

Males (N=22)				
Parameter	$\bar{x}$ ± SD	Min	Max	CV
Hair length (cm)	7,03±1,65	4,00	11,00	23,55
Hair diameter – shoulder (µm)	14,13±7,05	4,00	51,00	49,94
Hair diameter – back (µm)	14,50±6,99	4,00	51,00	48,25
Hair diameter – croup (µm)	15,32±7,65	3,00	51,00	49,97

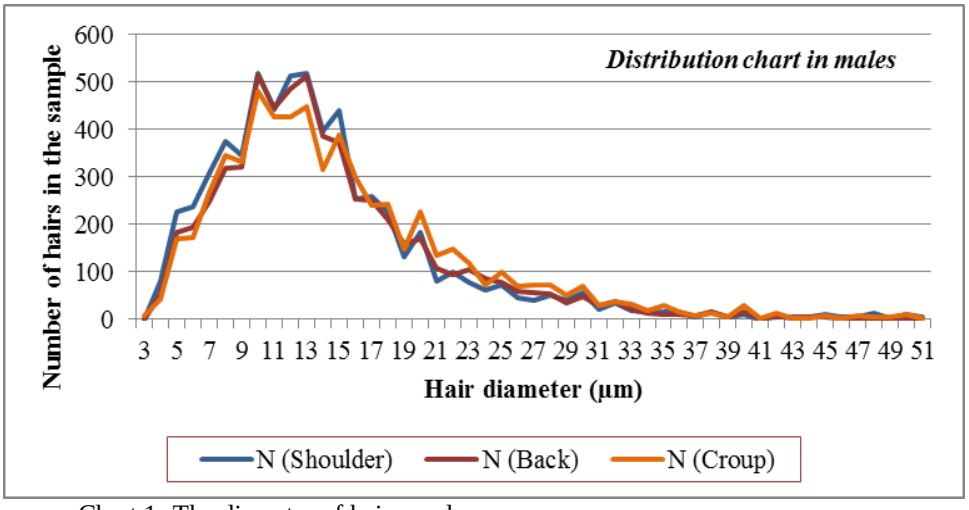

Chart 1: The diameter of hair - males

When it comes to the diameter of males` covercoat hair, the minimum diameter on the shoulders and the back was 4.00 µm (micrometer), while on the croup it was 3.00 µm. Maximum diameter of the hair was 51.00 µm. The average diameter of hair on the shoulders, the back and the croup in males was 14.13 µm, 14.50 µm, and 15.32 µm, respectively. The coefficient of variation is quite large in males, so for the shoulders it amounts 49.94%, for the back 48.25%, and for

the croup 49.97%. Standard deviation for the diameter of hair on the shoulders is 7.05, on the back 6.99, and on the croup 7.65. The hair cover in males had the largest diameter on the croup.

As shown on the above chart, the largest number of hairs in males had a diameter in the range between 4.00 µm and 21.00 µm from each body regions.

Table 2. Values of length and diameter of hair cover - females

Females (N=8)				
Parameter	$\bar{x}$ ± SD	Min	Max	CV
Hair length (cm)	6,67±1,23	4,00	10,00	18,47
Hair diameter – shoulder (µm)	14,71±7,04	3,00	49,00	47,92
Hair diameter – back (µm)	13,65±6,27	4,00	50,00	45,98
Hair diameter – croup (µm)	13,86±6,33	4,00	51,00	45,73

At female dogs, the hair on the shoulders had diameter of 14.71 µm on average, with standard deviation of 7.04. The corresponding values for the back amounted 13.65 µm with standard deviation of 6,27, and for the croup 13.86 µm with standard deviation of 6.33. The largest average diameter of hair in females was recorded on the shoulders.

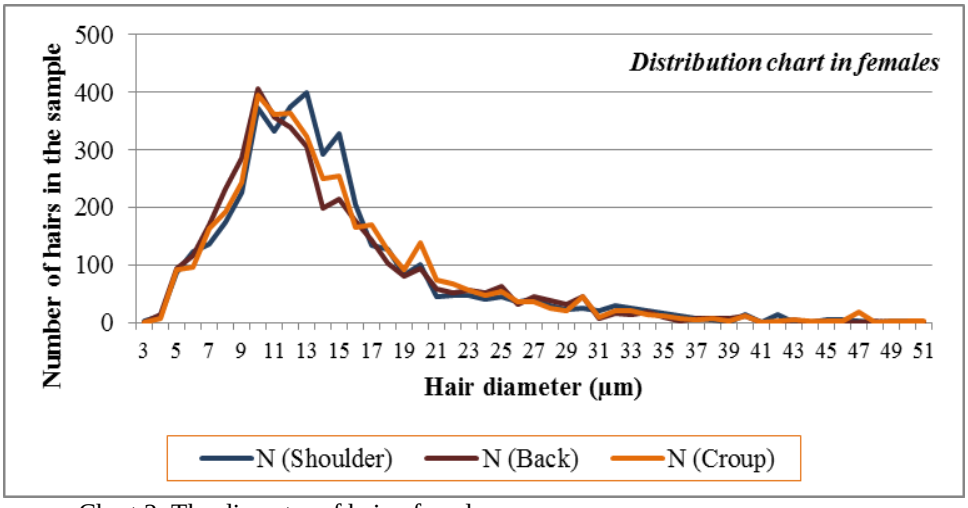

Chart 2. The diameter of hair - females

The standard describes the hair of the Barak as long, hard, shaggy, tousled, with a dense undercoat. Standard does not define the length of hair.

In the population of dogs that we examined, the average hair length was 7.03 cm in males, and in 6.67 cm in females. A quite large interval of variation is noticable, as well as cofficient of variation itself, which is 23.55% in males, and 18.47% in females. When observing the diameter of hair cover, we have noticed that the males` hair has the largest diameter on the croup (14.13 μm), while the females has the largest hair diameter on the shoulders, and it amounts 14.71 μm.

Comparing to other hound breeds in the region, we have found that the most similar hair cover has the Bulgarian barak (Urošević et al, 2018). When it comes to hair of this breed, the research show that average diameter of hair is 12.89 μm, with variation interval from 3.00 μm to 34.00 μm. The hair had the largest diameter on the croup, with average value of 14.00 μm, and variation interval from 3.00 μm to 30.00 μm. The hair had the smallest diameter on the shoulders. The average value was 11.94 μm with variation interval from 4.00 μm to 34.00 μm. Considering the values of diameter of covercoat hair for the Bosnian barak and Bulgarian barak, it is obvious that both breeds of dogs has coarse and hard hair (Urošević et al, 2018).

CONCLUSIONS

Bearing in mind that Bosnian broken-haired hound Barak is indigenous breed, working in the field enabled us to found a decrease in number of dogs, which can be a huge problem when it comes to preserving this breed in the long term. In the population involved in this study, the average hair length in males was 7.03 cm, and in females 6.67 cm. We have also found the large variation interval in both males and females, as well as coefficient of variation.

Based on the results obtained from this research, primarily the values of covercoat hair diameter, it can be concluded that this dog has coarse and hard hair, which completely justifies the very name of this breed (broken-haired).

This is the first analysis of the hair texture for the Bosnian broken-haired hound Barak, and the results can be used for further detailed description of this breed.

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Lenche VELKOSKA-MARKOVSKA, Biljana PETANOVSKA-ILIEVSKA[1]

DETERMINATION OF MALATHION IN PESTICIDE FORMULATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

SUMMARY

A new, simple, fast and reliable high-performance liquid chromatography (HPLC) method for determination of an active ingredient malathion in the pesticide formulation has been developed. Successful separation and quantification of malathion were achieved using Purospher STAR RP-18e (30 x 4 mm, 3 μm) and isocratic elution with mobile phase consisted of acetonitrile/water (47/53, V/V), flow rate of 1 mL/min, constant column temperature at 25 °C and UV detection at 220 nm. The specificity, selectivity, linearity, precision, accuracy, limit of detection (LOD) and quantification (LOQ) were tested for the method validation according to the CIPAC and SANCO guidelines. The obtained values for multiple correlation coefficients (R2 > 0.99), relative standard deviation (RSD) of retention times and peak areas (RSD ≤ 0.64%), and recoveries ranged from 101.04 to 101.84 %, revealed that the developed method has an excellent linearity, precision of retention time and peak area and accuracy. The proposed method was successfully applied for determination of an active ingredient malathion in the emulsifiable concentrate (EC) "Etiol techni" for a run time of 4 min.

Keywords: HPLC method, malathion, pesticide formulation, emulsifiable concentrate.

INTRODUCTION

Malathion is a broad-spectrum, non-systemic, organophosphorus insecticide and acaricide with contact, stomach and respiratory action. Products containing malathion are used to control a wide variety of insects in agriculture, as well in mosquito control, fly eradication and for treating lice (Gervais et al. 2020, Malathion 2020, Tomlin 1997).

Malathion is one of the most widely used insecticides in our country and in many other countries around the world. It has been approved for use according to European Commission Regulation (EC) No 1107/2009 (2009) and EPA (2009), etc. As an active substance, malathion could be found in many pesticide

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formulations, such as “Etiol techni”, which is in the form of emulsifiable concentrate (EC).

New pesticide formulations are being synthetized every day, and hence the need to control their quality is growing up. It is essential to enhance the quality of pesticide formulations placed on the market, thereby reducing inefficient control of pests, crop losses and risks to human health and the environment. Consequently, the simple, fast, precise and accurate analytical methods for determination of active substances in pesticide formulations are crucial to control the quality of pesticide formulations.

There are many analytical methods for determining malathion in different matrices and chromatographic methods are the most used, such as liquid chromatography (LC) (Torosyan et al. 2018a,b, Kara and Ince 2016), high-performance liquid chromatography (HPLC) (Hadjmohammadi et al. 2013, Ramin et al. 2019), high-performance thin layer chromatography (HPTLC) (Shayeghi et al. 2007), gas chromatography (GC) (Rezaee et al. 2019, Lofty et al. 2013, Khani et al. 2011, Bezerra et al. 2010, Singh and Dogra 2009) and gas-liquid chromatography (GLC) (Tomlin, 1997). Moreover, senzors (Cao et al. 2019) and spectrophotometric methods (Venugopal et al. 2013, Venugopal et al. 2012) are also used. Hardly a few methods for determination of malathion in pesticide formulations are known, among which Fourier transform infrared spectrometry method (Khanmohammadi et al. 2007). Furthermore, CIPAC (Collaborative International Pesticides Analytical Council) has been published reference methods for the determination of an active substance malathion in different pesticide formulations using gas-liquid chromatography (GLC) (CIPAC 1983) and gas chromatography (GC) (CIPAC 2003). Additionally, in the previous work, rapid resolution liquid chromatography (RRLC) method with ultraviolet diode-array detection (UV-DAD) for the determination of malathion in pesticide formulation has been described (Velkoska-Markovska and Petanovska-Ilievska 2019).

Moreover, the new analytical methods are always welcome to control the quality of pesticide formulations. Therefore, the aim of this work was to investigate the new possibilities for developing a simple, fast, precise and accurate high-performance liquid chromatography (HPLC) method for the determination of malathion as an active substance in pesticide formulation in the form of emulsifiable concentrate (EC) using ultraviolet-diode array detection (UV-DAD).

MATERIAL AND METHODS

Reagents and Chemicals

HPLC-grade acetonitrile and water, as well as, the Pestanal analytical standard of malathion (97.2% purity) were purchased by Sigma-Aldrich (Germany). The pesticide formulation “Etiol techni” was produced by “Galenika-fitofarmacija” (Belgrade, Serbia). It was in the form of an emulsifiable concentrate (EC) and declared values for the concentration and density were 600 g/L ± 25 g/L and 1.075 g/mL, respectively.

Equipment

The HPLC analyses were accomplished on an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) system equipped with: vacuum degasser (G1322A), binary pump (G1312B), autosampler (G1329B), a column compartment (G1316A), UV-VIS diode array detector (G1316B) and ChemStation software. The investigations were performed on a Purospher STAR RP-18e (30 x 4 mm, 3 μm) analytical column, produced by Merck (Germany). For the better dissolving of the stock and sample solutions an ultrasonic bath "Elma" was used.

Preparation of Standard Solutions

Stock solution of malathion was prepared by dissolving 0.0330 g of the pure analytical standard with acetonitrile in a 25 mL volumetric flask. The prepared stock solution was ultrasonicated for 15 minutes in an ultrasonic bath to achieve complete dissolution of the active component. The stock solution was stored in a refrigerator at 4°C. Under these conditions the stability of the active component was greater than one month.

Stock solution was used to prepare working standard solutions with different concentrations, by diluting the appropriate volume of stock solution with a mixture of acetonitrile and water (50/50, *V/V*) in 10 mL flasks.

A series of 5 working standard solutions with a concentration of 46.75, 93.50, 187.00, 280.50 and 374.00 μg/mL were prepared in order to test the linearity of the method. 5 μL of each of these working solutions was injected in triplicate.

A series of 8 working standard solutions in a concentration range of 66.74 ng/mL - 2672.5 ng/mL were prepared to determine the limit of detection (LOD) and the limit of quantification (LOQ). Each of these working solutions was injected three times with a volume of 5 μL.

Preparation of Sample Solution

Sample solution of pesticide formulation "Etiol techni" was prepared in a 10 mL volumetric flask by dissolving the weighed amounts of 0.0333 g in mixture of acetonitrile and water (50/50, V/V). The sample solution was degassed for 15 min in an ultrasonic bath. Afterwards, 0.5 mL from sample solution was transferred to a 10 mL volumetric flask and dissolved with a mixture of equal volumes of acetonitrile and water. The sample solution was completely dissolved in the solvents used and therefore there was no need to filter. Four injections were performed with a volume of 5 μL of this solution.

The recovery of the method was determinated by dissolving 0.5 mL from sample solution in three 10 mL volumetric flasks. In each solution was added a known amount of analytical standard of malathion: 23.37, 46.75 and 93.49 μg/mL. Then the flasks were supplemented to the mark with a mixture of acetonitrile and water (50/50, V/V). Four injections were performed with 5 μL of each of these solutions.

RESULTS AND DISCUSSION

The chromatographic studies were carried out using a short analytical column of type Purospher STAR RP-18e (endcapped), based on high purity, metal-free silica gel with polymeric C18 modification and endcapping. These columns

enable high efficient separations of base, neutral, acidic or chelating compounds using simple mobile phases, producing excellent peak symmetry. Thanks to remarkable stability over a wide range of pH values from 1.5 to 10.5 these columns allow the separation of complex samples using different mobile phases under different temperature conditions (ChromBook 2011).

Malathion (Fig. 1) is a generally accepted name according to ISO (International Organization for Standardization), while according to IUPAC (International Union of Pure and Applied Chemistry) the name of this active component is diethyl (dimethoxythiophosphorylthio)succinate; S-1,2-bis(ethoxycarbonyl)ethyl O, O-dimethyl phosphorodithioate) (Tomlin 1997).

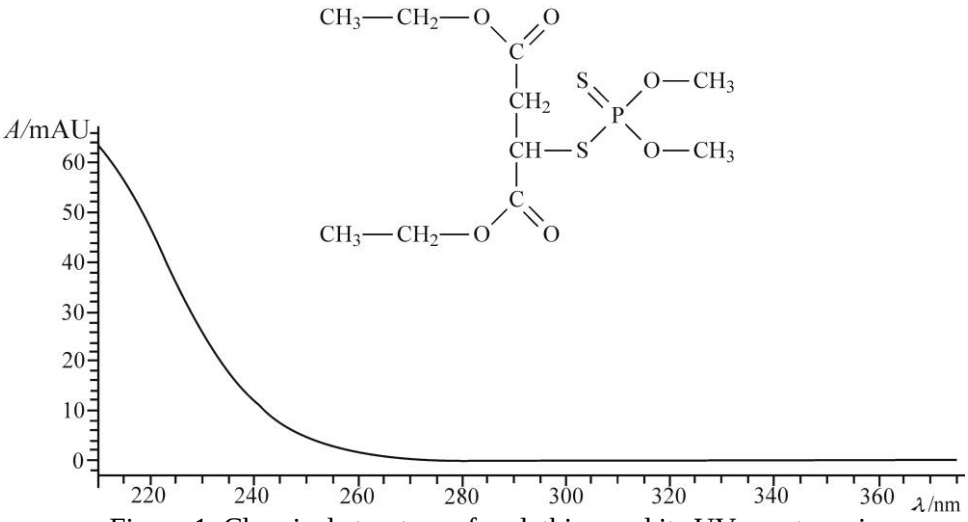

Figure 1. Chemical structure of malathion and its UV spectrum in acetonitrile/water (50/50, V/V)

On the basis of the UV spectrum of malathion recorded in acetonitrile and water solution (50/50, V/V) the wavelength was determined on which the chromatographic analyses were performed. As can be seen from the UV spectrum of malathion (Fig. 1), no maximum absorption was observed, but it was noticed that the absorption increases with decreasing wavelength. Therefore, the chromatographic analysis for the determination of malathion in the pesticide formulation was performed at 220 nm.

In order to obtain the optimum conditions for the determination of malathion in pesticide formulation, a series of preliminary tests have been performed by varying the volume ratio of acetonitrile to water in the mobile phase. In order to obtain a simple chromatographic method, isocratic elution was used. Studies have shown that the best conditions for determining malathion were obtained by using a mobile phase consisting of acetonitrile and water at a volume ratio (47/53, V/V) (Fig. 2), a flow rate of 1 mL/min, constant column temperature of 25°C and UV detection at 220 nm. At these chromatographic conditions, the dead time (t0) was

0.23 min and the malathion retention time (tR) was 2.58 min. Consequently, the calculated value for the retention factor (k') was 12.58, meaning less than 20, which is the maximum acceptable value for this parameter according to some authors (Dong 2006). At such defined chromatographic conditions of operation, a smooth base line and good peak shape of malathion were obtained. The value for malathion peak purity index was satisfactory (> 999). The time required for this analysis was approximately 4 min.

Figure 2b shows the chromatogram of the pesticide product "Etiol techni" obtained by the elaborate method. As can be seen from Figure 2b, the chromatogram of the pesticide formulation shows the presence of unknown components (X_1 and X_2) that eluted slower than malathion, with a retention time of 2.88 min and 3.20 min. Their chromatographic peaks were satisfying good separated to the baseline, and also from the malathion peak. That was confirmed by the calculated resolution values (RS = 2.56) and the separation factor (α = 1.14) of the chromatographic peak of malathion and its neighbouring peak (X1).

Specificity, selectivity, linearity, precision expressed as intra-day and inter-day repeatability of retention time and peak area, and accuracy were tested for the method validation according to CIPAC (2003) and SANCO rules (European Commission, 2019).

In addition, to confirm the specificity and selectivity of the proposed method, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index of malathion was greater than 999 (the maximum value for the peak purity index (PPI) should be 1000), which means that the chromatographic peak was not affected by any other compound. Furthermore, the identification of malathion in the pesticide formulation "Etiol techni" was performed by comparing the retention time of the analyte from the standard solution and from the sample solution, and confirmed by overlaying the absorption spectra of the pure analytical standard of malathion and the absorption spectra of the malathion in the pesticide formulation sample. The match factor value obtained by overlaid spectra was 999,918, which indicates that the peak was of the same substance.

A calibration curves were constructed to determine the linearity of the method, by plotting the injected amount of the standard of active ingredient as a function of the peak area and height, obtained by triplicate injection of 5 working solutions. The curves followed Beer's law in the concentration range from 46.75 μg/mL to 374.00 μg/mL (Table 1). Thereby, the maximum possible value of the multiple correlation coefficient (R^2) was obtained when the peak area was taken as the dependent variable (R^2=1), while the value of the multiple correlation coefficient when the peak height was taken as the dependent variable was significantly lower (R^2 = 0.9973). For these reasons, it was preferable that the calculations for the content of the active substance malathion in the pesticide formulation "Etiol techni" be performed according to the peak area. The results revealed excellent linearity of the proposed method.

Although, the limit of detection (LOD) and limit of quantification (LOQ) are not required according to CIPAC and SANCO guidelines, however, they were also determined.

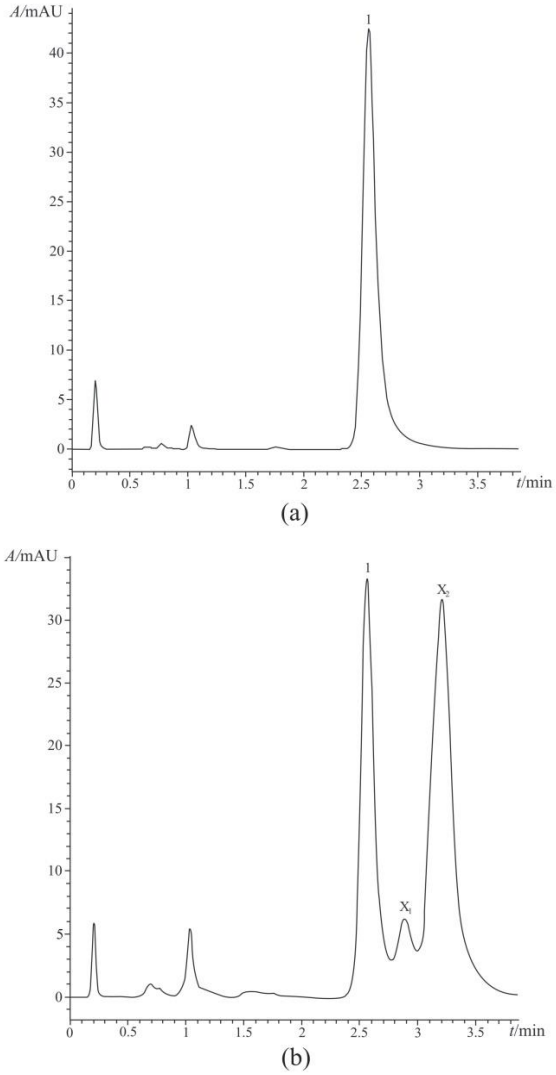

Figure 2. Chromatograms obtained from analytical standard of malathion (a) and pesticide formulation "Etiol techni" (b) on the Purospher STAR RP-18e (30 x 4 mm, 3 μm) at 220 nm using the proposed method

Although the new proposed chromatographic process was slightly longer (about 1.5 min), it could still be considered a fast and economic chromatographic method that requires insignificant higher consumption of organic solvent compared

to previously published results (Velkoska-Markovska and Petanovska-Ilievska 2020).

Figure 2b shows the chromatogram of the pesticide product "Etiol techni" obtained by the elaborate method. As can be seen from Figure 2b, the chromatogram of the pesticide formulation shows the presence of unknown components (X1 and X2) that eluted slower than malathion, with a retention time of 2.88 min and 3.20 min. Their chromatographic peaks were satisfying good separated to the baseline, and also from the malathion peak. That was confirmed by the calculated resolution values (RS = 2.56) and the separation factor ($\alpha = 1.14$) of the chromatographic peak of malathion and its neighbouring peak (X1).

Specificity, selectivity, linearity, precision expressed as intra-day and inter-day repeatability of retention time and peak area, and accuracy were tested for the method validation according to CIPAC (2003) and SANCO rules (European Commission, 2019).

In addition, to confirm the specificity and selectivity of the proposed method, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index of malathion was greater than 999 (the maximum value for the peak purity index (PPI) should be 1000), which means that the chromatographic peak was not affected by any other compound. Furthermore, the identification of malathion in the pesticide formulation "Etiol techni" was performed by comparing the retention time of the analyte from the standard solution and from the sample solution, and confirmed by overlaying the absorption spectra of the pure analytical standard of malathion and the absorption spectra of the malathion in the pesticide formulation sample. The match factor value obtained by overlaid spectra was 999,918, which indicates that the peak was of the same substance.

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Although, the limit of detection (LOD) and limit of quantification (LOQ) are not required according to CIPAC and SANCO guidelines, however, they were also determined.

The limit of detection (LOD) and the limit of quantification (LOQ) for malathion was determined by construction of a calibration curves in the low concentration region at 8 concentration levels (Table 1). The limit of detection (LOD) was calculated as three times the ratio between the SD and the slope of the low concentration curve (LOD = 3·SD/slope), and the limit of quantification (LOQ) as ten times the same ratio (LOQ = 10·SD/slope) (Miller and Miller 1993). The obtained values for LOD and LOQ are listed in Table 1.

Table 1. Statistical data for linearity, LOD and LOQ

Linearity range	Regression equation	R^2	SD	LOD (mg/L)	LOQ (mg/L)
46.75 - 374.00 µg/mL	[1]y = 551.1x + 0.0975 [2]y = 52.675x + 7.2254	1 0.9973	/	/	/
66.74 - 2672.5 ng/mL	[1]y = 2.2254x – 0.2396 [2]y = 0.2401x + 0.0207	0.9991 0.9992	10.3179 1.1113	3.06	9.26
[1]Area. [2]Height.					

The precision was expressed as day-to-day (n = 3) and within-day (n = 8) repeatability of retention time and peak area of malathion. For that purpose, eight successive injections of analytical standard of malathion with concentration 187.00 µg/mL, within 3 days (Table 2) were carried out. According to CIPAC and SANCO criteria, acceptable values for RSD were based on the modified Horwitz equation and they should not exceed 1.46 %. The RSD values obtained for the retention time (RSD = 0.08 – 0.30 %) and peak area (RSD = 0.08 – 0.64 %) of malathion were within acceptable limits. The results show that the proposed method was characterized by high precision of retention time and peak area.

Table 2. Statistical data for repeatability

	Intra-day repeatability (n = 8)						Inter-day repeatability (n = 3)	
	I day		II day		III day		$\bar{x}$ ± SD	RSD (%)
	$\bar{x}$ ± SD	RSD (%)	$\bar{x}$ ± SD	RSD (%)	$\bar{x}$ ± SD	RSD (%)		
Retention time (min)	2.58 ± 0.002	0.08	2.58 ± 0.004	0.15	2.57 ± 0.008	0.30	2.58 ± 0.006	0.22
Peak area	508.56 ± 1.08	0.21	515.77 ± 0.39	0.08	514.90 ± 0.73	0.14	513.08 ± 3.31	0.64

Compared to the previously published results (Velkoska-Markovska and Petanovska-Ilievska 2020), the precision of the proposed method was better. The accuracy of the method was confirmed by standard additions (CIPAC 2003, SANCO 2019). Accuracy of the method was expressed as the deviation between the calculated mean value obtained by examination and the true value of the spiked amounts of the analyte into a sample matrix that already contains some quantity of the analyte. The calculated values for the recovery were ranged from 101.04 to 101.84 % (Table 3). These values were within the acceptable values for the recovery according to the CIPAC (2003) and SANCO (2019) criteria, which should range from 98 to 102 %. Hence, it was concluded that the proposed method is accurate enough for determination of active ingredient malathion in the pesticide formulation "Etiol techni".

Table 3. Results from recovery experiments (n = 4)

Mass of analyte (μg)	Pure analyte added (μg)	Total analyte found (μg) (±SD)	Recovery (%)	SD (%)
0.48	0.12	0.61 ± 0.003	101.54	0.51
0.48	0.23	0.73 ± 0.0007	101.84	0.09
0.48	0.47	0.96 ± 0.0009	101.04	0.09

The proposed method was applied for the quantitative determination of the active component malathion in the pesticide product "Etiol techni". The obtained mean concentration of malathion was 616.55 g/L (n = 4, RSD = 0.20 %), which corresponded to the value declared by the producer. The experimentally obtained value for the density of the pesticide product was 1.069 g/mL.

CONCLUSIONS

This paper presents the new possibility for determination of an active ingredient malathion in the pesticide formulation "Etiol techni" using high-performance liquid chromatography method and ultraviolet - diode array detection. The short analytical column of type Purospher STAR RP-18e (30 x 4 mm, 3 μm) was used for identification and quantification of malathion. An isocratic elution with mobile phase consisted of acetonitrile/water (47/53, V/V), flow rate of 1 mL/min, constant column temperature at 25°C and UV detection at 220 nm was applied. The method validation was realized according to CIPAC and SANCO rules and showed that the developed method has an excellent linearity, precision of retention time and peak area, and accuracy. The obtained values for recoveries ranged from 101.04 to 101.84 %, with RSD of 0.09 – 0. 51 %, revealed that the proposed method is suitable for routine determination of malathion in the pesticide formulation in the form of emulsifiable concentrate for a run time of 4 min.

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Slađana GVOZDENOVIĆ, Dragana DRAKULOVIĆ and Milica MANDIĆ [1]

SEASONAL DYNAMIC OF POTENTIALLY TOXIC AND TOXIC PHYTOPLANKTON AND BIOTOXINS ON MUSSEL FARM (*Mytilus galloprovincialis* Lamarck, 1819) IN KAMENARI – BOKA KOTORSKA BAY

SUMMARY

Results about toxic and potentially toxic phytoplankton species and biotoxins are given in this paper. Investigation is conducted on mussel (*Mytilus galloprovincialis* Lamarck, 1819) farm situated in Kamenari (Tivat Bay) from January 2015 up to September 2016. In total six harmful phytoplankton taxons are identified, with clear seasonal variation in their abundance. *Pseudo-nitzchia* spp. were the most abundant (maximum abundace 1.1×10^5 cells/l), while *Prorocentrum micans* and *P. cordatum* reached maximum abundance up to 10^3 cells/l during some seasons. Other toxic taxons had low abundance, while in some seasons were completely absent. Results about quantitative and qualitative analysis of biotoxins showed that all obtained values were below limit of detection (LOD), what implies on good quality of mussels meat on investigated farm, safe for human consumption.

Integrated monitoring of harmful phytoplankton and biotoxins should be continued in order to prevent possible negative consequences caused by increased growth of harmful algae (harmful algal blooms – HABs) and biotoxins.

Key Words: harmful phytoplankton, biotoxins, mussels, Boka Kotorska Bay, Adriatic Sea

INTRODUCTION

Boka Kotorska Bay is situated on southeastern part of Adriatic Sea. The Bay can be devided in three entities: Kotor-Risan Bay, Tivat Bay and Herceg Novi Bay (Mandić S. *et al*., 2016). It is semi-enclosed aquatorium with specific hydrography compare to open waters. Surrounded by karst Mountains (Orjen and Lovćen) the Bay is during winter and spring under influence of fresh water, what significantly affects physical, chemical and biological characteristic. Inflows of freshwater in Boka Kotorska Bay are numerous: rivers, springs, streams and

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submarine springs. Currents in the Bay depend on meteorological conditions (primary winds) and freshwater inflow from the coast and underwater sources (Bortoluzzi *et al*., 2016). Currents on the surface mainly depend on winds, while currents on the bottom layer depend on freshwater inflows (Bellafiore *et al*., 2011).

It is known that changes in physical and chemical parameters of water significantly affect phytoplankton. Temperature, salinity as well as nutrients (SiO_4^-, PO_4^{3-}, NO_3^-, NO_2^-, P, N) are among most important parameters affecting quantitative and qualitative phytoplankton composition (Drakulović, 2012; Pestorić *et al*., 2019).

Diatoms and dinoflagellates are two main phytoplankton groups in marine ecosystems with crucial role in marine ecosystems as primary producers. There are about 300 phytoplankton species considered as harmful (Arapov, 2013). In general, regarding the effect they cause, harmful phytoplankton species can be observed as: high-biomass producers and toxin producers (Arapov, 2013). Phytoplankton toxins, also known as biotoxins, are associated with harmful algal blooms (HABs). HABs are influenced by a number of factors, including climate change and nutrient inputs from anthropogenic activities (Watson *et al*., 2015; Brooks *et al*., 2016). Due to increasing eutrophication around the world, HABs are occurring in more locations than ever before, and in contrast to large-scale blooms that are dominated by mesoscale circulation, Mediterranean HABs are more localized phenomenon, commonly related to areas of constrained dynamism, such as bays, lagoons, ports, beaches and estuaries (Ferrante *et al*., 2013).

Biotoxins can cause serious consequences and even death to aquatic organisms and humans (Visciano *et al*., 2016). Based on symptoms they cause in humans, biotoxins can be divided in six groups: diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), azaspiracid shellfish poisoning (AZP) and cuguatera fish poisoning (CFP) (Arapov, 2013; Gvozdenović *et al*., 2015). Depending on their solubility, biotoxins can be classified as hydrophilic and lipophilic, where hydrophilic toxins involve ASP, PSP and the emerging pufferfish poisoning (PFP), while the lipophilic toxins are associated with DSP, AZP, as well as emerging CFP, NSP and cyclic imines (CIs) (Estevez *et al*., 2019). Europe Union legislation set regulatory limits for marine biotoxins (EC No. 853/2004; 854/2004; 15/2011, 786/2013).

Aquaculture is the fastes growing food production sector in the world, which can be serious affected by HABs and biotoxins. Because of that, different monitoring programmes (HABs and biotoxins monitoring) has been established worldwide.

In Montenegro exist about 20 farms all situated in area of Boka Kotorska Bay, where Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) is dominant farming species (Mandić M. *et al*., 2016; Gvozdenović *et al*., 2017). Monitoring of qualitative and quantitative composition of toxic and potentially

toxic phytoplankton on farms is continuously conducted by the Institute of Marine Biology, University of Montenegro, while biotoxins monitoring is continuous conducted by Center for Eco- Toxicological Research. This integrated monitoring give the possibility to react on time in order to prevent possible negative consequences caused by HABs and biotoxins.

The aim of this paper is to present results about seasonal dynamic of harmful phytoplankton and biotoxins on mussel farm in Kamenari.

MATERIAL AND METHODS

Investigation has been conducted from January 2015 up to September 2016 on mussel farm in Kamenari – Boka Kotorska Bay (Figure 1).

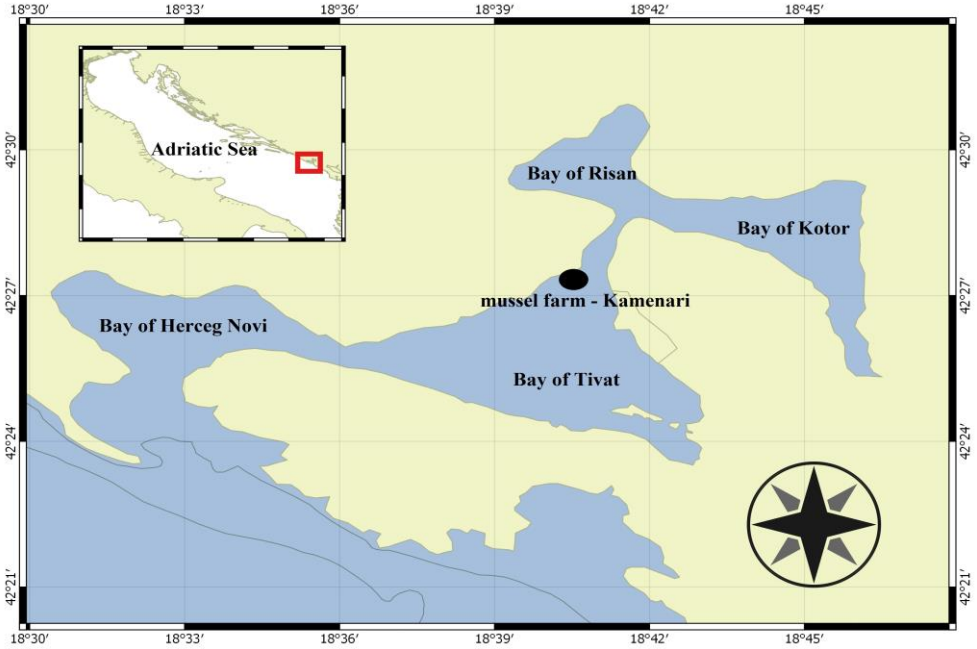

Figure 1. Map of Boka Kotorska Bay – black circle shows mussel farm situated in Kamenari

The farm is situated on the entrance of strait Verige which connect Kotor-Risan and Tivat Bay. Water sampling was done by ISO 5667-9:1992. Samples for analysis of temperature and salinity were taken by Niksin bottle between 2 and 3 m depth, each month since January 2015 to September 2016. Temperature and salinity were measured *in situ* by CTD probe (Multiline P4; WTW). Samples for phytoplankton analysis were also taken by Niksin bottle between 2 and 3 m depth, seasonaly (winter 2015, spring 2015, summer 2015, autumn 2015, winter 2016, spring 2016, summer 2016). Phytoplankton analysis were done by standard method (MEST EN 15204:2014). Samples were preserved in 4% formaldehyde. In laboratory, samples were dropped in 25 ml chambers for sedimentation during 24 hours. Quantitative analysis (cells counting) were done using Leica DMI4000

B inverted microscope in subsamples of 25 ml (Utermöhl, 1958). Qualitative analysis involved using of keys for phytoplankton determination (Cupp, 1933; Hustedt, 1930; Peragallo and Peragallo, 1965; Dodge, 1985; Schiller, 1933; 1937; Sournia, 1989).

Mussels for biotoxins analysis were sampled monthly in line with Europe laboratory for marine biotoxins protocol. About 2 kg of mussels was sampled, soft tissue was separated from shell and frozen until analysis. ASP toxin analyse (domoic acid) was done according to Quilliam *et al*. (1995) protocol using HPLC-UV-DAD, while PSP toxins (STX, dcSTX, GTX1,4, GTX2,3, NEO, C1,2) were analysed according to AOAC (2005) protocol using HPLC-FLD. Limit of detection (LOD) was calculated based on the standard deviation of the blank samples.

RESULTS AND DISCUSSION

Temperature and salinity values of sea water are given on Figure 2. The minimum temperature was observed during January 2016 (10.5 °C), while the maximum temperature was measured during August 2015 (26.5 °C). Average water temperature during investigated period was 18.7 °C. Temperatures below 15 °C were present during winter months, while temperatures above 20 °C were observed during summer months what is in accordiance to the other results obtained for the Boka Kotorska Bay (Drakulović *et al*., 2013; 2014; 2015).

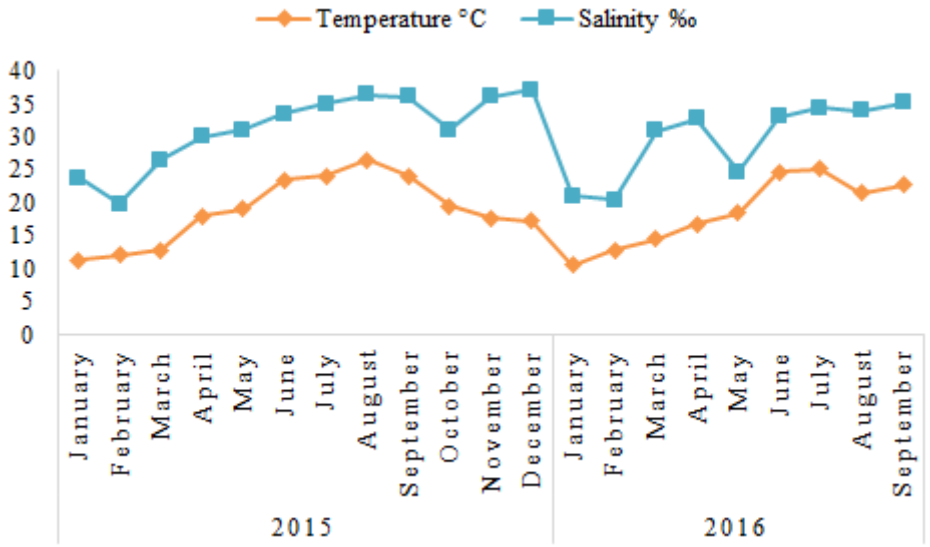

Figure 2. Temperature and salinity value of water on mussel farm in Kamenari during investigated period

Water salinity ranged from minimal value during February 2015 (19.8 ‰) up to maximum value during December 2015 (37.1 ‰). High salinity was also

present during summer period. Average water salinity during investigated period was 30.6 ‰. Salinity is abiotic factor strongly affected and depended on freshwater inflow; higher salinity is mainly present during summer, while lower salinity is present during winter. Obtained results are in accordiance with other results obtained for the Bay (Drakulović *et al*., 2013; 2014; 2015), although maximum salinity value during December 2015 is not usual. These „extreme“ can be explained by quite warm and dry period during November and December 2015 with absence of rain.

Abundance of toxic and potentially toxic phytoplankton is presented on Figure 3. In total six taxons are identified. Five species belonged to dinofllagelates (*Prorocentrum cordatum, P. micans, Phalacroma rotundatum, Lingulodinium polyedra* and *Dinophysis acuminata*), while one taxon belonged to diatoms (*Pseudo-nitzchia* spp.). Results show seasonal variations in phytoplankton abundance. *Pseudo-nitzchia* spp. was the most abundant during all investigated seasons, except season winter 2016, when this taxon was absent. Maximum abundance of this taxon was during summer 2015 when reached values 1.1×10^5 cells/l. *Prorocentrum cordatum* and *P. micans* reached values up to 10^3 cells/l during spring 2016 and summer 2016, respectively. *Phalacroma rotundatum, Lingulodinium polyedra* and *Dinophysis acuminata* had low abundance ranged from 40–80 cells/l, while during most seasons were absent.

Biotoxins analysis indicate absence of domoic acid and saxitoxins in mussles tissue, all obtained results were below LOD. LOD for all seven analysed biotoxins is given in Table 1.

Phytoplankton blooms are not just result of increased nutrients, other abiotic factors like temperature and freshwater inflow can also be a reason (Price *et al*., 2015; Ninčević Gladan *et al*., 2020). Dinoflagellates are the group that includes the largest number of harmful species which can cause blooms and biotoxins production. Drakulović (2012) indicated that abundance of toxic dinoflagellates in area of Boka Kotorska Bay is not alarmant.

Although results showed that *Prorocentrum micans* and *P. cordatum* did not exceed abundance above 10^3 cells/l, Drakulović *et al*. (2012) reported abundance of *P. micans* in Boka Kotorska Bay in range of 10^6 cells/l. For the same area, Bosak *et al*. (2011) indicate *P. cordatum* as dominant species in phytoplankton assemblage, with a maximum abundance 3.97×10^4 cells/l.

All five dinoflagellate species identified in this research are known to produce toxins as ocadaic acid and dinophysisitoxins, which can cause DSP in humans, as well as pectenotoxins and yessotoxins wich adverse effect in humans has not yet been confirmed (Arapov, 2013).

Pseudo-nitzchia spp. are known as dominant phytoplankton taxons among central and south Adriatic Sea (Burić *et al*., 2008). *Pseudo-nitzchia* spp. and *Nitzschia* spp. produce domoic acid which cause ASP in humans (Arapov, 2013; Gvozdenović *et al*., 2015). Ujević *et al*. (2010) indicate that toxic species do not always express toxicity, and if the abundance does not reach 1.0×10^5 cells/l, then the area can be considered safe with respect to ASP.

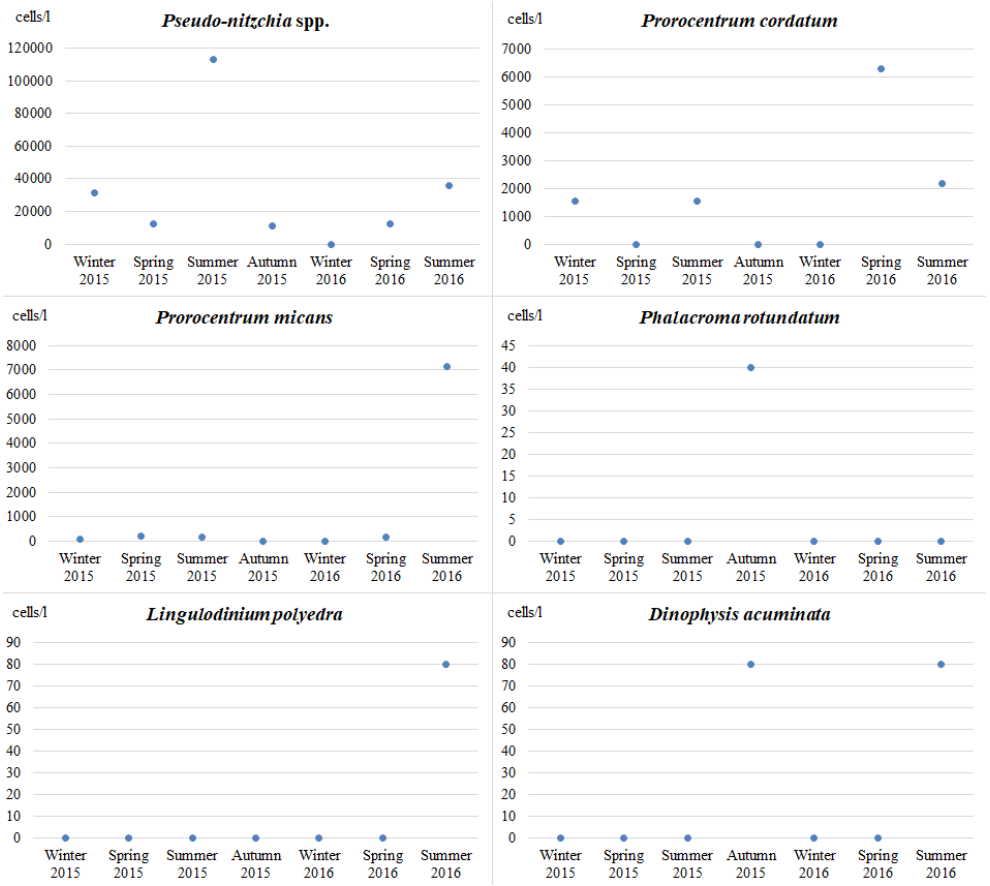

Figure 3. Seasonal dynamic of potentially toxic and toxic phytoplankton taxons on mussel farm in Kamenari

Table 1. LOD for all seven biotoxins

Biotoxin	LOD
Domoic acid (mg/kg)	0.326
STX (µg/kg)	51.42
dcSTX (µg/kg)	1.168
GTX 1,4 (µg/kg)	55.5
GTX 2,3 (µg/kg)	8.368
NEO (µg/kg)	43.9
C 1,2 (µg/kg)	7.368

In this paper abundance of *Pseudo-nitzchia* spp. above 1.0×10^5 cells/l was just during summer 2015. Also in all mussel samples domoic acid was below LOD. Arapov *et al*. (2017) identified five potentially toxic species from genus *Pseudo-nitzchia* in central Adriatic Sea: *P. calliantha*, *P. delicatissima*, *P.*

fraudulenta, P. pseudodelicatissima/ P. cuspidata and *P. subfraudulenta*, while in area of Kotor Bay, Bosak *et al*. (2010) identified *P. calliantha*. The same authors consider that blooms of this species appear in Kotor Bay.

Species from genus *Alexandrium*, as well as *Gymnodinium catenatum* and *Pyrodinium bahamense* are known to produce saxitoxins (Arapov, 2013; Gvozdenović *et al*., 2015). Toxic phytoplankton species which can produce saxitoxins have not been identified during investigation on farm in Kamenari. Additionally, those species have not ever been identified for Boka Kotorska Bay area (Drakulović, 2012; Drakulović *et al*., 2017). Obtained results are also supported by negative results of saxitoxins, as all six investigated saxitoxins in all mussel samples were below LOD.

Results of this paper are in accordiance to the results which are given by Pestorić *et al*. (2019) for mussel and fish farm in Orahovac which is also situated in area of Boka Kotorska Bay.

CONCLUSIONS

HABs can cause problems in marine ecosystems such as hypoxia and anoxia, as well as biotoxins producing which further accumulate in shellfish. On this way biotoxins became part of food chain and cause serious consequences, even fatal, in marine organisms and humans. Results obtained in this paper indicate that abundance of toxic and potentially toxic phytoplankton taxons is not alarmant, as well as biotoxins values bellow LOD, what implies on good quality of mussels meat on farm in Kamenari. Monitoring of harmful phytoplankton composition and biotoxins should be continued in future, so it can give the possibility to react on time in order to prevent negative consequences which can be caused by HABs and biotoxins.

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ANTIOXIDANT CAPACITY AND CHEMICAL COMPOSITION OF FIVE IRANIAN WILD STRAWBERRIES (*Fragaria vesca* L.)

SUMMARY

Strawberry is small fleshy fruit, with high values of biochemical contents. The essential effects of strawberry in human health have been approved due to significant amount of its vitamin C, antioxidant, phenolic compounds and anthocyanin. Wild strawberry (*Fragaria vesca* L.), from Rosaceae family, grows wildly in some north regions of Iran and have been traditionally used for food and medicinal purposes. In this study, five wild strawberry populations from Gilan province in the north of Iran, were used to investigate the value of chemical traits including; total soluble solid (TSS), titratable acidity (TA), vitamin C and bioactive content; antioxidant activity, total anthocyanin and total phenolic content. Among these five populations, Masoleh population showed the highest value of assessing factors while Ardeh population had the lowest. The total phenolic content was statistically correlated to the antioxidant activity ($r = + 0.99$; $p < 0.05$) and total anthocyanin content ($r = +0.93$; $p < 0.05$). Correlation matrix of biochemical contents showed that the climate condition (altitude, longitude, latitude) of collected fruits can be related to the biochemical quantity. According to the results, high concentration of the antioxidant activity was presented in the Iranian wild strawberries. Current study suggests that environmental factors can change the antioxidant capacity and biochemical compositions of wild strawberry. To sum it up, wild strawberry populations of Iran can be considered as a good source for breeding program, plus it may be introduced as a fruit with great value for human health.

Keywords: Biochemical compounds, Small fruits, Diversity, Cluster analysis, Correlation

INTRODUCTION

Strawberry, a high-value berry crop, is grown in many temperate and sub-tropical regions of the world. The strawberry is small fleshy fruits, which are commercially cultivated and commonly consumed in fresh and processed forms (Seeram et al., 2008).This tasty fruit classified in Fragaria genus. The Fragaria

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genus consists of at least 20 species and belongs to Rosaceae family (Hummer et al., 2011; Liston et al., 2014). Diploid wild strawberry (*Fragaria vesca* L.) grows naturally throughout Europe, North America and North Asia (Sillasoo, 2006; Ozcan and Haciseferogullari, 2007). Worth to mention, it grows wildly in north regions of Iran especially in Mazandaran and Gilan proveniences (Figure 1) and has been used as food and medicinal remedies in folk medicine in this region of Iran.

Figure 1. Wild Strawberry in the Masoleh region (Gilan province)

Biochemical contents such as vitamin C, are one of the most important nutritional quality factors in strawberries (Du et al., 2009). Strawberries as well as blackberries, and raspberries are rich in antioxidant, phenolic compounds and anthocyanin and the high amounts of these biochemical contents propound essential effects of strawberries in human health in many ways (Herrmann and Nagel, 1989; Macheix et al., 1990).

Several studies have been reported about the biochemical compounds in different species of strawberry. Biochemical compositions have been reported in Fragaria × ananassa (Kafkas et al., 2007; de Souza et al., 2014). Furthermore, the antioxidant capacity of strawberry has been reported in different strawberry species such as: *Fragaria* × *ananassa* (Wang and Lin 2000; de Souza et al., 2014) and *Fragaria vesca* L. (Dyduch-Siemińska et al., 2015).

As our knowledge, there is not sufficient information about wild diploid strawberry populations in Iran. This study is the first report which reveals

biochemical properties and bioactive compositions in Iranian wild strawberries. Therefore, we aimed to investigate the native origin variations in five *Fragaria vesca* L. populations of Gilan province in north of Iran. In current study, biochemical properties including; total soluble solid, titratable acidity, total anthocyanin, total phenolic content, vitamin C and antioxidant activity of Iranian wild strawberry populations were evaluated. Soil properties of collected populations in mentioned regions were analyzed in relation to the biochemical compositions of wild strawberry populations.

MATERIAL AND METHODS

Sample collection

Five wild strawberry populations (1kg for each population) were collected from different regions of Gilan province in North of Iran from June to July 2016. These strawberries populations are day neutral and collected in growing period.

All samples were collected from their native origin and there were no treatments on them. The names of populations come from sample collection (Sangdeh, Ardeh, Ponel, Zendaneh and Masoleh). The geographical properties of collected sample region including latitude, longitude and altitude were determined by the GPS and are shown in Table 1. Also some of climatic characteristics belonged to the nearest weather station to the studied regions are presented in Table 2.

Table 1. Location information of five regions that samples were collected

Region	Latitude (N)	Longitude (E)	Altitude (m)
Sangdeh	53°13'	36°40'	1273
Ardeh	48°49'	37°32'	1018
Ponel	49°60'	37°31'	1054
Zendaneh	48°44'	37°32'	1720
Masoleh	48°59'	37°90'	1289

Table 2. Climate condition parameters during in the months of growing to collected samples (2016)

	April	May	June	July
Max. Temp (°C)	29.8	29.2	32.1	33.2
Min. Temp (°C)	3	7.4	14	17.5
RH (%)	77	78	73	68
Precipitation (mm)	110.3	34.5	48.20	40.4
The average wind speed (m/s)	20	8	11	9
The average sunshine hours (h)	86.8	122.8	190.9	220.6
The average of temperature (°C)	13.4	18.2	22.2	25.5

Determination of Chemical fruit characteristics

Total soluble solid was measured by using a digital refractometer (Erma, Tokyo, Japan; calibrated using distilled water). Titratable acidity was measured by following the official method of AOAC (1984).

Determination of bioactive contents

Vitamin C content was determined by using 2, 6-dichlorophenol indophenol dye according to the method described by Ruck (1963). Briefly, a juice sample (30 g) was homogenized in 500 ml of 0.4% oxalic acid. After infiltration, 20 ml of the mixture was tittered with the dye and finally results were expressed as mg/100 g juice.

Total anthocyanin content was determined using the pH-differential method (Muanda et al., 2011). The pH values of the diluted pulp juice were 1.0 (in 0.025 M potassium chloride buffer) and 4.5 (in 0.4 M sodium acetate buffer), respectively. As a brief, in this method, 400 μL from extract solution was mixed separately 3.6 mL with each buffer. Using a spectrophotometer, absorbance was measured at 510 nm and at 700 nm. The amount of absorption is calculated as follows: $A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$

Total phenolic contents were estimated as gallic acid equivalent by using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965). The extracted juice (1 mL) was mixed with 5 mL Folin-Ciocalteau reagent, and then 4 mL sodium carbonate (7.5% w/v) was added. The combination was diluted to 100 mL with distilled water and was kept in the dark at room temperature for 2 h. the absorbance of the mixture was measured at 765 nm with spectrophotometer. Gallic acid was used as standard and the concentration of total phenolic compounds in the extracts were expressed as galic acid equivalent g^{-1} extract.

Antioxidant activity was assessed using the DPPH method and have strong absorption over the wavelength 520 nm (Thaipong et al., 2006). In the present study, for measuring the antioxidant activity, 2 ml of 5, 10, 20, and 40 (ppm) plant juice concentrations was diluted by 2 ml of DPPH (100 μM methanol solution) and mixture was taken at room temperature for 30 minutes. The absorbance was measured at 520nmwith spectrophotometer.

Determination of soil properties

Determination of physical and chemical soil properties of studied regions were figured out by using the standard laboratory procedure and presented in Table 2. After transferring the samples to laboratory, they were air-dried and passed through a 2 mm sieve. Soil samples were analyzed to determine particle-size distribution (hydrometer method), pH (measure of acidity), EC (saturated extract at 25 $^{\circ}$C), Moisture (%)and SOC (%) (Soil organic carbon percent). Also, total nitrogen (N), phosphor (P), potassium (K) and Sodium (Na), in saturation extract were assessed (Miransari et al., 2008).

Data analysis

The experiment was a completely randomized design with three replications. The normality test was done using the Kolmogorov–Smirnov test

and data were analysed by the MSTATC software (version 1.2, Michigan State University, East Lansing, MI).

Comparison of means carried out by Duncan's multiple range test. Differences were considered significant at the level of$p \leq 0.05$. Pearson's correlation coefficients (r) were calculated using Microsoft Excel 2010 software.Cluster analysis using the Ward's method was also carried out using SPSS statistical software (version. 21.0 for Windows) to classify and group the population according to their biochemical properties.

RESULTS AND DISCUSSION

In Table 3, it can be observed the results of soil properties in studied regions. Results of analysis of variance indicated that there were highly significant differences (p<0.05) among samples for all parameters (Table 4). Also, some important biochemical characteristics of Iranian wild strawberry populations are summarized in Table 5.

Table 3. Physical and chemical properties of soil in studied regions

Region	Soil texture	N (%)	P Av. (mg/kg)	K Av. (mg/kg)	Moisture (%)	Organic carbon (%)	EC (dS/m)	pH
Sangdeh	Loam	0.15	12	180	42	1.4	3.3	6.1
Ardeh	Clay	0.14	13	190	51	1.3	1.6	6.5
Ponel	Loam	0.17	12	260	62	1.6	1.6	6.7
Zendaneh	Loam	0.17	12	160	67	2.12	2.2	6.3
Masoleh	Loam	0.18	12	250	71	2.24	1.3	6.2

Table 4. Analysis of variance for biochemical and bioactive composition of Iranian wild strawberry populations

S.O.V	df	Total soluble solid	Titratable acidity	Total phenolic content	Total anthocyanin content	Vitamin C	Antioxidant activity
Region	4	1.73**	0.26**	3905333.08**	0.49**	101.74**	34.33**
Error	10	0.03	0.007	0.55	0.005	0.07	0.53
CV (%)	-	2.26	6.05	0.01	2.21	0.92	1.77

S.O.V: Source of variation, d.f: degree of freedom, C.V: coefficient of variation and ** significant at 1% probability levels

Table 5. Means of biochemical and bioactive compositions of some Iranian wild strawberry populations

Origin	Total soluble solid (Brix)	Titratable acidity (%)	Total phenolic content (mg/100 g)	Total anthocyanin content (mg/100 g)	Vitamin C (mg/100 g)	Antioxidant activity (%)
Sangdeh	7.7^{b}	1.50^{b}	574.75^{b}	3.36^{b}	35.32^{a}	43.17^{b}
Ardeh	7.2^{c}	1.03^{d}	463.20^{e}	2.96^{d}	22.24^{d}	37.04^{e}
Ponel	7.3^{c}	1.50^{b}	512.78^{d}	3.13^{c}	26.46^{c}	38.82^{d}
Zendaneh	8.7^{a}	1.23^{c}	522.20^{c}	3.02^{cd}	28.12^{b}	40.32^{c}
Masoleh	8.7^{a}	1.80^{a}	757.51^{a}	3.95^{a}	35.66^{a}	45.50^{a}

Values in the columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan multiple range test.

Chemical properties

In present study, the amounts of total soluble solid (TSS) value varied between 7.2 °Brix (Ardeh population) to 8.8 °Brix (Zendaneh and Masoleh populations). Titratable acidity varied from 1.03 to 1.80% in Ardeh and Masoleh populations, respectively.

Vitamin C contents of present study were between 22.22 mg per 100 g (Ardeh population) to 35.66 mg per 100 g (Masoleh population). The vitamin C levels in this studywere lower than the value in strawberry species (*Fragaria* × *ananassa*), blackberry (*Rubus spp)*, red raspberry (*Rubus idaeus)* and blueberry (*Vaccinium corymbosum)* (deSouza et al., 2014; Celik et al., 2018). These differences may be related to their genetic sources, soil property, as well as growing condition.

In terms of total anthocyanin content, this parameter ranged between 2.96 mg per 100 g in Ardeh population to 3.95 mg per 100 g in Masoleh population. Total anthocyanin contents of wild Iranian strawberry populations were higher than other *Fragaria vesca* L. cultivars (Baron von Solemacher, Yellow Wonder and Regina) cultivars which reported by Dyduch-SiemiNska et al. (2015).

Total phenolic contents varied from 463.20 to 757.51 mg GAE per 100 g in Ardeh and Masoleh populations, respectively. Total phenolic contents in this study showed higher valuesrather than other *Fragaria* species; *Fragaria* × *ananassa* (Wang and Lin 2000; Hakala et al., 2003; Pantelidis et al., 2007; de Souza et al., 2014) and *Fragaria vesca* L. (Dyduch-Siemińska et al., 2015). Different climatic conditions and growth conditions can be affected by biochemical properties in strawberries species (Hakala et al., 2003).

Antioxidant activity value assessed as 37.04% in Ardeh population and 45.50% in Masoleh population. The result of this study was supported by other studies in *Fragaria* species (deSouza et al., 2014;Kafkas et al., 2007).

As a brief, the highest values of chemical compositions and antioxidant capacity were observed in Masoleh population, while the lowest rank was seen in Ardeh population (Table 2). The diploid species of the *Fragaria* genus have been adapted to their localized environments (Hancock and Luby, 1993). The chemical compositions and antioxidant capacity depends on genetic factor as well as environmental factors (Mohammadi and Asadi-Gharneh., 2018).

In comparison to the result of this study and the literature, the analysed fruits presented high concentrations of antioxidant activity (Table 6). Present study demonstrates Iranian wild strawberry population have enormous nutritional value and are considered important as a valuable germplasm. Also the high value of antioxidant capacity in the wild strawberries, as shown in previous studies in this field, may reduce the risk of developing cancer and heart diseases (Ascherio et al., 1992; Renaud and Lorgeril 1992; Wargovich, 2000).

Correlation analysis

Correlation coefficients among all measured biochemical traits in the wild diploid strawberry populations are given in Table 7.

In current study, vitamin C is clearly positive correlated with total anthocyanin content ($r = 0.97$; $p< 0.05$) as well as titratable acidity ($r = 0.83$; $p< 0.05$), total phenolic content($r = 0.83$; $p< 0.05$) and antioxidant capacity($r = 0.82$; $p< 0.05$). There is several reports about relationships between the antioxidant activity and the phenolic compounds and anthocyanin contents in strawberry (deSouza et al., 2014) as well as berries and cherries (Hassimotto et al., 2008; Koca and Karadeniz 2009; Wu et al., 2010). Current observation supported by the previous researches reported in berries and cherries fruits (deSouza et al., 2014).

Correlation matrix of biochemical contents showed that the climate condition (Altitude, longitude, latitude) of collected plants can be related to the biochemical contents. In this study altitude had the positive correlation with total soluble solid ($r = +0.83$; $p< 0.05$). Also longitude and latitude had negative ($r = -0.93$; $p< 0.05$) and positive ($r = +0.86$; $p< 0.05$) correlation with EC of the soil, respectively. Furthermore, moisture of soil had the positive ($r = +0.87$; $p< 0.05$) and negative ($r = -0.76$; $p< 0.05$) correlation with longitude and latitude, respectively. The effect of climate and altitude on physiochemical traits of different populations is the notable point (Khakdaman et al., 2007). Climatic conditions have considerable effects on biochemical properties of wild populations (Mohammadi and Asadi-Gharneh., 2018; Javanmard et al., 2018).

The total phenol content was positively correlated to the antioxidant activity($r = +0.99$; $p< 0.05$) and anthocyanin content($r = +0.93$; $p< 0.05$). Anthocyaninsclassified in phenolic compounds which are water-soluble pigments responsible for the orange, red and blue colour of many fruits (Mazza and Miniati 1993). Therefore, this strong correlation between the phenol contents and anthocyanins can be expected.

Table 6. Comparison ofchemical and bioactive components of strawberry species.

Reference	Species	Vitamin C (mg/100 g)	Total soluble solid (Brix)	Titratable acidity (%)	Antioxidant activity (%)	Total phenolic content (mg/100 g)	Total anthocyanin content (mg/100 g)
Kafkas et al., (2007).	*Fragaria annanasa*	-	6.33–10.86	0.60–1.31	-	-	
Wang and Lin, (2000)	*Fragaria annanasa*	-	-	-	12.2-17.4	95-152	2.3-4.5
Hakala et al., (2003)	*Fragaria annanasa*	32.4-84.7	-	-	-	-	-
Pantelidis et al., (2007)	*Fragaria annanasa*		-	-	-	-	-
de Souza et al., (2014)	*Fragaria annanasa*	90.1	10.5	0.9	37.8	621.92	1.6
Dyduch-SiemiNska et al. 2015	*Fragaria vesca*	-	-	-	11.2-15.3	164-284	0.9-1.6
Current study	*Fragaria vesca*	22.2 -35.6	7.2-8.7	1.0-1.8	37.0-45.5	463.2-757.5	2.9-3.9

Table 7. Correlation coefficients between all measured biochemical traits in the wild strawberry population

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1- Latitude	1.00															
2- Longitude	**-0.89**[a]	1.00														
3- Altitude	-0.11	0.02	1.00													
4- N	-0.34	0.61	0.42	1.00												
5- K	-0.18	0.52	-0.56	0.52	1.00											
6- P	-0.32	0.07	-0.51	-0.75	-0.23	1.00										
7- Moisture	**-0.76**	**0.87**	0.38	**0.87**	0.42	-0.36	1.00									
8- Organic carbon	-0.49	0.66	0.67	**0.88**	0.17	-0.57	**0.88**	1.00								
9- EC	**0.86**	**-0.93**	0.30	-0.40	-0.63	-0.28	-0.71	-0.36	1.00							
10- pH	-0.44	0.28	-0.52	-0.04	0.44	0.32	0.17	-0.30	-0.56	1.00						
11- Antioxidant activity	0.08	0.37	0.02	0.56	0.51	-0.45	0.35	0.55	-0.18	-0.54	1.00					
12- Total phenolic content	0.00	0.42	0.17	0.64	0.44	-0.50	0.45	0.66	-0.19	-0.57	0.99	1.00				
13- Total anthocyanin content	0.31	0.09	0.33	0.54	0.20	-0.65	0.23	0.57	0.18	-0.78	0.91	0.93	1.00			
14- Total soluble solid	-0.27	0.40	**0.83**	0.68	-0.15	-0.54	0.64	**0.92**	-0.04	-0.62	0.53	0.66	0.68	1.00		
15- Titratable acidity	0.24	0.23	0.01	0.70	0.65	-0.73	0.36	0.50	-0.08	-0.34	**0.90**	**0.88**	**0.85**	0.41	1.00	
16- Vitamin C	0.51	-0.14	0.31	0.43	0.13	-0.70	0.05	0.42	0.38	**-0.80**	**0.82**	**0.83**	**0.97**	0.57	**0.83**	1.00

[a] Bold digit showed the high positive and negative correlation.

Cluster analysis

Cluster analysis based on studied biochemical properties, grouped the wild strawberry population into two main groups with a respective distance of 10 out of 15 (Figure 2). Lambda statistic of Wilks was done for determining cutoff point in cluster analysis.

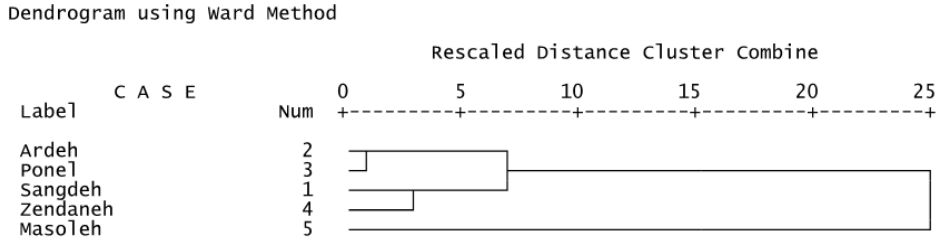

Figure 2. The cluster analysis of the wild strawberry populations according to the biochemical traits using the Ward's method

The first cluster containing 4 wild populations which are divided to two sub-cluster. Ardeh and Ponel populations were placed in one sub-cluster as well as Sangdeh and Zendaneh. In the first sub-cluster, the average of traits including total soluble solid, total phenolic content, vitamin C and antioxidant activity were less than other population and have the average levels of biochemical properties. The populations of Sangdeh and Zendaneh were in the second sub-cluster and in terms of studied traits; higher amounts were reported in comparison rather than other sub-cluster. Finally, Masoleh population was placed in a separate cluster that is defined with high level of studied traits. In current study; Ardeh and Masoleh population had the most distance. In general, some differences in biochemical characteristics affected the separation of wild population in to different clusters.

CONCLUSIONS

In the present study, we evaluated the antioxidant capacity and chemical composition of some wild Iranian strawberry populations. There are significant differences among the biochemical properties of wild strawberry populations. Masoleh population was assessed as the highest value of antioxidant activity while Ardeh population was in the lowest rank. This variation in wild populations can be exploited by breeders to expand of this plant as a source of natural bioactive compounds that could replace with the synthetic antioxidant. Concluding, this that environmental factors may have influence on antioxidant capacity and biochemical compositions of wild strawberry.

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RISK ASSESSMENT OF TOXIC ELEMENTS (Pb, Cd, Hg, As) IN WATER, SEDIMENT AND IN THINLIP GREY MULLET (BOJANA RIVER)

SUMMARY

The Bojana River is outflow of the Skadar Lake. River is shared by Albania and Montenegro, it forms part of their border before flowing into Adriatic Sea. During most of the year the water quality of the Bojana River is acceptable, but pollution increases the most frequently in summer period.

For this study, research of toxic elements (Pb, Cd, Hg, As) in the water and sediment of the Bojana River, as well as in the muscle tissue and digestive tract tissue of thinlip grey mullet (*Chelon ramada*) was done at the end of the summer season. This research of toxic elements in muscle tissue and digestive tract tissue of thinlip gray mullet is done for the first time for this species, as well as bioconcentration factor (BCF), biota sediment accumulation factor (BSAF) and Spearman correlation between the matrices. The grade of metals concentrations decreased in the subsequent order: As>Pb>Cd=Hg in water and As>Pb>Cd>Hg in sediment. The order of decreasing concentration for metals in muscle tissue is as follows: Hg>Pb=Cd=As and in digestive tract tissue of thinlip grey mullet: Pb>Hg>Cd=As. The highest values of BCF in muscle tissue of fish have been established for Hg and Cd and in digestive tract for Pb and Hg. The values of biota sediment accumulation factor (BSAF) decreased in the following order: Hg>Cd>Pb=As for muscle tissue and Hg>Pb>Cd>As. for digestive tract tissue.

Key words: Bojana River, thinlip grey mullet, toxic elements, BCF, BSAF

INTRODUCTION

Bojana is a large river (5.187 km^2) and is located in the 5th Ecoregion, i.e the Dinaric Western Balkans region and its length of river flow belonging to Montenegro (27.5 km). It is proposed to be one water body and belongs to type 9 - large area basin, valley (altitude <200 m), geological characteristics limestone-organic. The hydrological characteristics of the Bojana River depend on the hydrological characteristics of Skadar Lake and its tributaries, and especially of the River Drim, which flows into Bojana. Drin River (Drinisa) with the Lake

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Skadar outflow (Bojana-Buna) the total catchment comprises about 19.600 km^2 and the average discharge is 672 m^3/s (Schneider-Jacoby, 2006, Buric et al., 2019). The Bojana is the border River between Montenegro and Albania. The bottom of the riverbed is between 2 to 5 meters below sea level. Although the course of the Bojana River is relatively short, the hydrological regime of this river is extremely complex. The Skadar (Shkoder) Lake and Bojana (Buna) River system, with its delta area on the Adriatic Sea, contains important ecosystems with fresh and brackish water. The Bojana Buna River mouth represents a rare example of a natural delta on the East Adriatic coast (Fanelli et al., 2015).

Thinlip grey mullet, *Chelon ramada* (Risso, 1827) is frequently observed in large shoals throughout coastal areas, brackish water and entering also freshwater. According to the IUCN red list of threatened species is classified as least concern. The wide distribution and success of this species is attributed to their high euryhaline capacity (Turan, 2015; Freyhof and Kottelat, 2018).

Many factors influence speciation of elements in freshwaters. Evaluation of elements speciation in waters is a complex task. The more soluble forms of "heavy" metals are considered as more bioavailable and toxic (De Paiva Magalhães et al., 2015). With the assumption that heaviness and toxicity are inter-related, term "heavy metals" also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Tchounwou et al., 2012). Big impact on the water quality of the Bojana River has waste water from Skadar (Albania) and Ulcinj (Montenegro), as well as unsolved contamination of Port Milena (Ulcinj). The Bojana River links marine and freshwater ecosystems being a fish migration corridor, moving from the Adriatic Sea to Skadar/ Shkoder Lake (Fanelli et al., 2015).

The aim of this research is to analyse four toxic elements (Pb, Cd, Hg, As) in Bojana River water and sediment, as well as, in muscle tissue and in digestive tract tissue of thinlip grey mullet, with BCF and BSAF assessment.

MATERIAL AND METHODS

Study area. All samples were collected from Bojana River (Fig.1). The sampling sites were near to the Bojana River delta area ($19^0 20" 58.61"$ E, $41^0 52" 12.00"$ N). The water samples (of 1 l), were bottled and saved in hand held refrigerator and transferred to the laboratory. The sediment samples were taken by Ekman dredge, from the depth of 0–20 cm, packed in plastic boxes and transported to the laboratory. The samples of fish were taken from local professional fishermen, packed in plastic bags and transported to the laboratory by hand held refrigerator.

The analysis of water samples of the Bojana River (Pb, Cd, Hg, As), as well as muscle and digestive tissue samples of thinlip gray mulet was done in the Chemical Laboratory of the Institute of Public Health, while the sediment analysis at the Eco-Toxicological Center (CETI).

The content of elements in water samples (previously acidified to pH <2) were directly determined using the ICP-OES technique (induced coupled plasma-

optical emission spectrometer, Spectro ARCOS). Standard solutions of Pb, Cd, Hg and As concentration 1000 mg/l (BT Baker) were used for preparation of calibration solutions. Operating wavelengths and PQL values for the analysed elements are given in Tab.1

The analysis of the elements content in the muscle and digestive tissue of fish was determined in the wet tissue of fish. In parallel, the fish tissue was dried at 105^0 C, and then treated in the same way as the wet tissue of the fish, in order to determine the normalized content of elements in them. Preparation of samples for analysis of metal content in fish tissue was done in accordance with the standard method (Analytical Method Perkin Elmer of AAS).

Table 1. Operating wavelengths and practical limits of quantification

Metals	Working wavelength	PQL (mg/l)
Pb	283.3	0.05
Cd	228.8	0,001
Hg	225.6	0.0005
As	224.5	0.0005

Figure 1. Bojana River outflow of Skadar Lake, inflow Adriatic Sea

Regarding the sediment analysis, 0.3-0.5 g of the sample was taken and transfer to a digestion vessel. Then, 9 ml of concentrated nitric acid and 3 ml of concentrated hydrochloric acid were added. The vessel was closed and placed in

the microwave oven for digestion. After the digestion, the cooled solution from the vessels (with filtration) was transferred to the volumetric flask (25 ml) and made up to the mark with deionized water. Following methods and instrumental techniques were used: Pb by AOAC 985.01 method, instrumental technique AAS; Cd by AOAC 982.23 method, AAS; Hg by CETI 1 method, Hg analyser; As by EPA 3051A* (accredited).

The concentrations of elements in water are given in *mg/l*,while in sediment and in fish muscle tissue and digestive tract tissue are given in *mg/kg*.

Statistical analyses

Bioconcentration factor (BCF). Several terms are used to define the concentration of elements in aquatic biota. BCF is defined as the net result of the absorption, distribution, and elimination of a particular substance in biota that has entered through water (Mc Geer et al., 2003). Bioconcentration factor is defined as the uptake of contaminants from the dissolved phase. It can be calculated by the following equation: $BCF=C_B/C_W$. where C_B represents the average concentration of the element in the biota i.e a certain tissue (μg/g of most mass), and C_w concentration of the element in water (μg/ml) (Mc Geer et al., 2003; Mackay and Fraser, 2000; Pollman and Axelrad, 2014; USEPA, 2015).

Biota sediment accumulation factor (BSAF*)* is defined as the concentration of contaminant uptake by biota from sediment: $BSAF=C_B/C_W$, where C_B represents the average concentration of the element in the biota ie a certain tissue (μg/g) and C_S refers to the concentration of the element in the sediment (μg/g) (Mackay and Fraser, 2000; Pollman and Axelrad, 2014; USEPA, 2015).

Statistical analyses are applied in the study, as well as Spearman's (rho) coefficient of correlation. Spearman's rho rank correlation coefficient is denoted by the symbol r_s for the sample data and the p_s symbol by the base set data. This correlation coefficient is the simple linear correlation coefficient between ranks.

There is no strictly standard rule for assessing the strength of a relationship, but most often in use is: weak: 0 - 0.25, moderate: 0.25 to 0.60 and strong: 0.60 to 1. The smaller sample, the higher the coefficient required to achieve statistical significance. It is important to note that the degree of correlation between two variables is not static, but can swing over a wide range from inverse to positive and vice versa, over the time (Dodge, 2010; *Myers et al, 2003)*. Statistical analysis has been done in R statistical computing software, Version 3.5.3. for Windows.

RESULTS AND DISCUSSION

Heavy metals are well-known environmental pollutants due to their toxicity, persistence in the environment, and bioaccumulative nature. Metal pollution from multivarious sources has adverse effects on aquatic ecosystems (Ali, 2019; Perera et al., 2015).

Pollution of aquatic ecosystems with toxic elements is an environmental problem of public health concern. Being persistent,pollutants accumulate in the environment and consequently contaminate the food chains. Elements that pose

the highest risks for human health are: mercury, cadmium lead and arsenic, which cause important complications. Trophic transfer of these elements in aquatic food chains, has important implications for wildlife and human health (USEPA, 2015). Among a wide range of toxic substances that contaminate fish, three heavy metals; cadmium, lead and mercury are the only heavy metals included in the European Union regulations for hazardous metals (Perera et al., 2015).

Pb in water and sediment of Bojana River and in thinlip grey mullet

Lead is the most important toxic heavy element in the environment. According to its physico-chemical properties, its use can be retraced to historical times. Globally it is an abundantly distributed, important yet dangerous environmental chemical. Its important properties like softness, malleability, ductility, poor conductibility and resistance to corrosion seem to make difficult to give up its use. Accordingly to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards (Wani et al., 2015). Lead gets to the aquatic system due to the ground superficial erosion and atmospheric deposition. Environmental levels have increased over 1,000 times in the last three centuries as result of human activity (Rodriguez et al., 2015). Human exposure to lead and its compounds occurs mostly from various sources: industrial processes such as smelting of lead and its combustion, pottery, boat building, lead based painting, lead containing pipes, battery recycling, grids, arm industry, pigments, printing of books, etc (Wani et al., 2015). Most of the toxic metal pollutants are adsorbed by the suspended particles in water. These adsorbed metals undergo complex migration and transformation processes in the water–sediment–organism, such as adsorption, desorption, precipitation, biological absorption, and other reactions (Yi, 2020).

By analysing the concentration of toxic elements in the water of Bojana River, lead is at the second place (Fig. 2). The order of decreasing concentration of toxic elements in water of Bojana River is as follows: As>Pb>Cd=Hg, as well as in sediment: As>Pb>Cd>Hg. The determined lead values i sediment were 7±1 mg/kg. In digestive tract tissue of thinlip gray mullet determined values were 0.01, while in the digestive tract have been established value of 2.54 mg/kg (Fig. 2). In accordance with the Regulation on the maximum level for certain contaminants in food (ILA, 2018, Annex, Part III Metals), the allowable MPC (mg/kg wet weight) in fish muscle tissue is 0.30. The correlation coefficient between water and sediment is 0.949 ($p = 0.051$) as shown in Fig.5.

In the aquatic environment lead is primarily bound in sediment, under the low pH and along with number of other factors becomes more bioavailable. In freshwater and marine ecosystems, the accumulation of lead is insignificant and considered as non-dangerous for humans by using fish in the diet, except in cases of large and extraordinary pollution. According to the sediment quality guidelines (SQGs) of USEPA concentrations of metals is mostly lower than the possible effect level (PEL), (Yi, 2020; USEPA, 2000, 2007). Consequently, sediment quality has emerged as an important and critical consideration for protection of

benthic ecosystem health, fisheries conservation, and protection of surface water quality in both freshwater and marine environments (Babut et al., 2005; Wenning et al., 2005, Kvok et al., 2014).

According to MPC of toxic substances in sediment due to Dutch and Canadian recommendations), lead values obtained in this study (7±1) are much lower (Fig.2) than the quality standards and recommendations for sediment, according to Dutch methodology, target value (85.mg/l), Netherlands Government Gazette, 2000; and from Canadian legislation (theoretically possible impact value (35 mg/kg), Canadian sediment quality guidelines, 2001.

Fish among the group of aquatic organisms represent the largest and most diverse group of vertebrates. A number of characteristics make them experimental models for toxicological research, especially for the contaminants which are likely exert their impact on aquatic system.

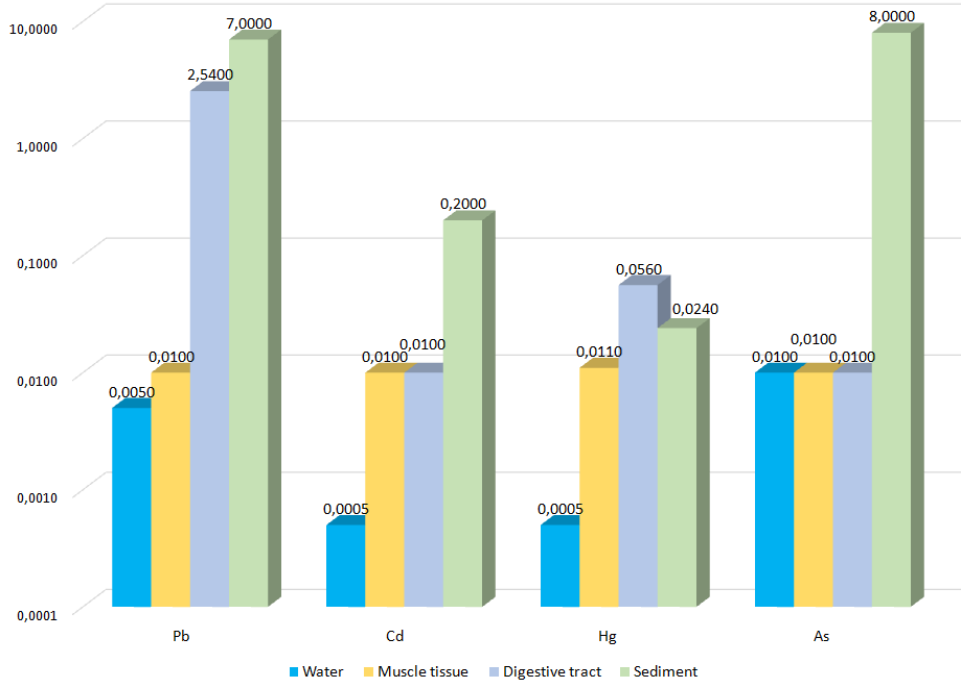

Fig 2.Concentration of Pb, Cd, Hg and As in water, sediment, muscle and digestive tissue

Since not all chemical forms of pollutants are equally bioavailable and some pollutants can be accumulated in living organisms to a greater extent than others, we need to study the levels of pollutants in the organisms to be able to predict the *environmental risk* (Yancheva et al., 2015).

The concentration of lead in the digestive tissue of thinlip gray mullet is 2.54 mg/kg (Fig.2), while the value of bioconcentration factor in digestive tract

tissue is 508 (Fig. 3). Biota sediment accumulation factor shows lower values (Fig.4).

Cd in water and sediment of Bojana River and in thinlip grey mullet

Cadmium and its compounds are very toxic, although their toxicity was established only in the first half of the last century. Cd is persistent in freshwater ecosystems for many years, while in the oceans it is assumed that it can remain for thousands of years. It ranks seventh on the list of 20 most important toxins (ATSDR, 2007). Cadmium is not subject of biomethylation processes, but it accumulates to a large extent (Agbaba, unpublished article). It has a long biological half-life.

Figure 3. BCF of Pb, Cd, Hg and As in thhilnip grey mullet (muscle and digestive tract tissue)

The concentration of cadmium in the sediment of the Bojana River is at the third place between the toxic elements (Fig. 2): As>Pb>Cd=Hg. The presence of cadmium in the water of the Bojana River is in accordance with the concentration as mercury. The value obtained in this study (0,2 mg/kg Cd) are much lower than the quality standards and recommendations for sediment, according to Dutch

methodology and Canadian legislation, Dutch methodology-reference target value is 0.8 mg/kg, and intervention value:12 mg/kg; Canadian recommendations ISQG (0.6 mg/kg), PEL (3.5), ICPDR (1.2 mg/kg). According to the EU directive 466/200 and Regulation EC, No 333/2007, the permitted cadmium (Cd) value in fish meat is 0.1 mg/kg. The values determined by this study are lower (Fig.2).

Authors Neziri and Gössler, 2006 had analysed Bojana River water and established Cd concentration value of <0.1, while in the sediment they determined Cd value of 27.3 mg/kg. These values are higher than the values determined in this research (Fig. 2).

The established value of BCF for cadmium in muscle tissue, as well as in the digestive tract of thinlip grey mullet is 20 (Fig.3). Biota sediment accumulation factor shows lower values (Fig.4). Deviations in BSAF values (biota, sediment, accumulation factor) predicted with fission models may be due in part to differences in the bioavailability of chemicals, which may lead to marked variation of specific sites, in the bioaccumulative profiles of certain pollutants (Van der Oost et al., 2003).

Hg in water and sediment of Bojana River and in thinlip grey mullet

Mercury can arise in the environment from natural sources such as volcanic eruptions, soil erosion and bacterial decomposition of organic mercury compounds and anthropogenic sources such as: municipal waste incinerators, fossil fuel, combustion factory, electrolysis factory (here mercury is used as an electrode). Elemental mercury and its salts in the environment, mainly are result due to direct emissions from industry, while organo-metallic mercury which can also occur in the environment, is most often the result of pesticides (e.g. mercury-based fungicides), The basic problem of mercury pollution is that its organometallic compounds can accumulate and metabolize in the biosphere (Sofilic, 2014).

Similar to non-metals and in contrast to most other metals, mercury (Hg) forms organic compounds that are very stable in the environment. All forms of mercury are potentially toxic but the levels of toxicity vary considerably (Agbaba, unpublished article).

The intake of metals through food in comparison with the direct absorption from the solution is of fundamental importance in heterotrophic aquatic organisms, because in the polluted aquatic environment the intake of dietary excess i.e, trophic levels is higher than the intake through water, due to richer metal content in sediment, particles and detritus (Sharmeen et al., 2014). The concentration value of mercury in the water of the Bojana River is the same as for cadmium (Fig. 1).

Authors Neziri and Gössler (2006), analysing mercury in the water of Bojana River determined value of <0.1 (μg/l), while in sediment <0.5 mg/kg. The stated value for sediment is higher than the value determined in this study, where determinate value is in the concentration of 0.024 mg/kg (Fig. 2).

Contamination of sediments is mostly dangerous because of how it "travels up" the food chain, ultimately becoming harmful to animals and humans (USEPA, 2000: EPA, 2007).

In relation to quality standards and recommendations for sediment, according to Dutch methodology, Canadian legislation, as well as ICPDR, Dutch methodology, reference value is 0.3 mg/kg, Canadian recommendations PEL value is 0.486, ICPDR: 0.8 mg/kg, and there values are higher than ISQG value: of 0.17 mg/kg (Babut et al., 2003; Canadian sediment quality guidelines, 2001; Kvok et al, 2014). The values obtained by this study are much lower in relation to quality standards and sediment recommendations, according to Dutch methodology, Canadian legislation, as well as ICPDR.

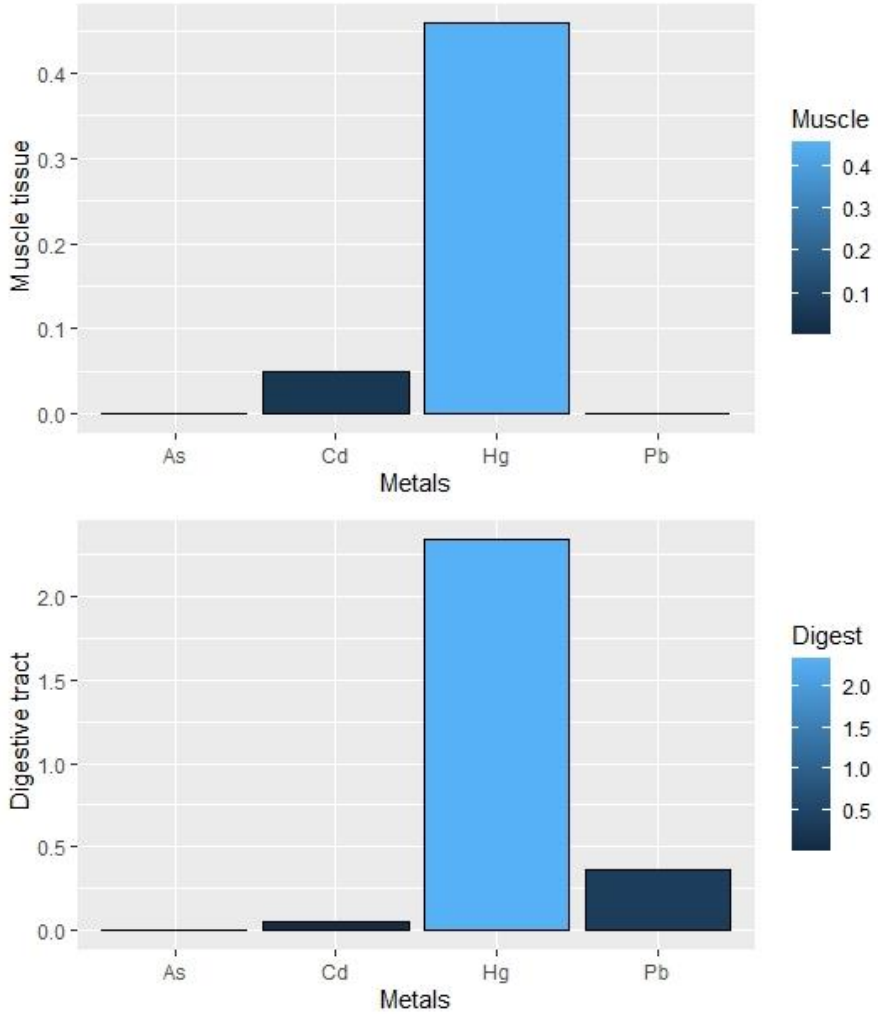

Figure 4. Biota sediment accumulation factor (BSAF of Pb, Cd, Hg and As in thhilnip grey mullet)

The established concentration of mercury in the tissue of the thinlip gray mullet digestive tract was 0.056 mg/kg (Fig.1), value of bioconcentration factor 112 and the determined values of BCF in muscle tissue of thinlip gray mullet 22. (Fig, 3). Consistent with the bioconcentration of toxic metals in this study, mercury was found to be in the first place in terms of bioconcentration in muscle tissue of thinlip gray mullet (Hg>Cd>Pb>As). BSAF values show much lower values, although the established dominance of biota sediment accumulation factor were also observed both in muscle tissue and in the digestive tract: Hg>Cd>Pb>As, (Fig. 4).

The concentration of toxic elements: Pb, Cd, Hg and As in water of Bojana River are less than the limit, established by the EU directive 75/440 EEC (European Environment Agency, EPA, 2007).

Elements Pb, Hg, Cd are the most important from the ecotoxicological aspect since they are not essential (they do not have a known metabolic function) and their presence in the body is exclusively a consequence of contamination. They have a high ratio of anthropogenic and natural intake in the body. Pb, Hg, Cd are endocrine disruptors and immunosuppressants (Agbaba, unpublished article). Fish have been found to be good indicators of water contamination in aquatic systems because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates are also more sensitive to many toxicants (Yancheva et al., 2015).

As in water and sediment of Bojana River and in thinlip grey mullet

Arsenic is often found in ores of silver, tin, lead, cobalt, copper and nickel. The ores are not mined for arsenic, but it is obtained as a by-product in the isolation of ores of other metals such as copper, lead, cobalt and gold. Oxide ores of which the most important is arsenolite (As_4O_6) are much rarer (Petrak and Pavlovic, 2015).

With the production of protective chemicals for wood, then herbicides, insecticides, the USA stood out as the largest consumer of As until 2003, after which the use decreased. Herbicides with As are allowed in the USA, while EU regulations recommend avoiding chemicals that contain As, and pesticides based on As are prohibited. High concentrations of arsenic (As) have been reported for water samples in several parts of the world and over 200 million persons globally are at risk of arsenic exposure at levels of concern for human health. The United States Environmental Protection Agency (USA EPA) sets limits for arsenic concentration in freshwaters to protect aquatic organisms from arsenic. It has established criteria maximum concentrations (CMC) and criteria continuous concentrations (CCC) for most inland surface waters. According to the US EPA, the CMC for acute arsenic exposure is 340 μg As/l, while the CCC for chronic arsenic exposure is 150 μg As/l (Barral Fraga, 2020).

Arsenic distribution between the water column and the sediment is controlled by several physico .chemical and biological processes, such as precipitation, solubilisation, adsorption, desorption, oxidation, reduction,

incorporation in the crystal structure of minerals, and biological exchanges. Arsenic is ranked the first of all hazardous substances by the Agency for Toxic Substances and Disease Registry (Barral-Fraga, 2020). As is found in waters such as seawater, warm springs, groundwater, rivers, and lakes. In aquatic environments, As occurs as a mixture of arsenate and arsenite, with arsenate usually predominating.

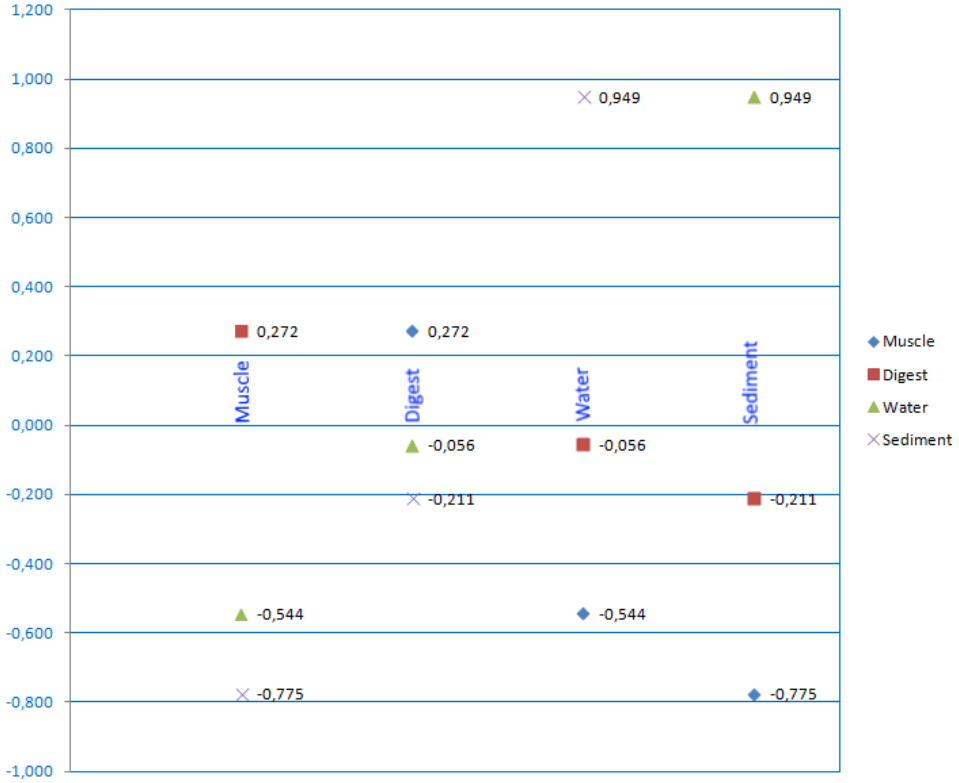

Figure 5. Spearman's correlation coefficient between matrices

The concentration of arsenic in water, as well as in the sediment is in the dominant first position (Fig. 2): As>Pb>Cd=Hg. Arsenic levels in Pampean surface waters have been attributed to the hydrogeology of the streams, fed by an aquifer with high levels of arsenic (0.6 to 4.9 mg/l) originated from quaternary loess sediments (Barral Fraga, 2020). Geochemical investigations showed that most arsenic in the sediments of the Anllóns River is associated with low-mobility phases specifically as bound to Fe-oxide forms and in the residual phase. Arsenic levels in natural systems are often well below those that cause mortality in higher organisms such as fish, but even low concentrations may impede normal functioning. Arsenic is toxic in all four oxidation states (+V, +III, 0, -III), and trivalent and pentavalent arsenic are the most common found in ecosystems (Rahman et al., 2014).

The concentration of arsenic of 9.6 mg/kg in the sediment of Bojana River was determined by the authors Neziri and Gössler (2006). The stated value for sediment are higher than the value determined in this research, which is 8±1 mg/kg (Fig. 2). In this research, in terms of bioconcentration, arsenic is at the fourth place Hg>Cd>Pb>As.

Continuous exposure of freshwater organisms including fish to low concentrations of As results in bioaccumulation, notably in liver and kidney. As a consequence induces hyperglycemia, depletion of enzymatic activities, various acute and chronic toxicity and immune system dysfunction (Kumari et al., 2016).

Arsenic is persistent in the environment and risk for biota and human health. This metalloid enters the biota in primary consumers and then biomagnifies to higher order organisms (Alvarado-Flores et al., 2019).

Concentrations of total arsenic in uncontaminated nearshore marine and estuarine sediments usually fall in the range of 5 to 15 μg/g dry weight. Concentrations of total arsenic in whole or muscle tissues of marine organisms worldwide range from below the detection limit of the analytical method, usually 0.01–0.6 μg/g dry weight (depending on the method). Pollution of aquatic ecosystems with toxic elements is a worldwide problem because of the environmental persistence and biomagnifications in food chain (Ali et al., 2019; Flores et al., 2019).

CONCLUSSION

The influence of toxic elements and the associated risk assessment is in accordance with the physico-.chemical and biological conditions in the aquatic ecosystem. The concentration of toxic elements: Pb, Cd, Hg and As, decreased in the subsequent order: As> Pb> Cd=Hg in the water of the Bojana River and As>Pb>Cd>Hg in sediment. The order of decreasing concentration for metals in muscle tissue is as follows: Hg>Pb=Cd=As and in digestive tract of thinlip grey mullet: Pb>Hg>Cd=As. The highest values of BCF in muscle tissue of thinlip grey mullet has been for Hg and Cd, while in digestive tissue for Hg and Pb. The values of BCF decreased in the following order: Hg>Cd>Pb>As in muscle tissue, as well as in digestive tract.

Transfer through the food chain of these metals could have consequences for aquatic biota and for human health and it is of great importance to assess and monitor the concentrations of these potentially toxic elements and their bioavailability.

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RECULTIVATION OF DEGRADED SOIL DUE TO MINING ACTIVITY WITHOUT ADDING ORGANIC LAYERS OF SOIL USING ALFALFA AND MIXTURES OF GRASS LEGUMES

SUMMARY

The aim of this research was to examine the possibility of reparing the chemical properties of substrate and regenerating the vegetation of degraded soil due to mining activity without adding the fertile soil layer. The research was carried out in the period 2011-2013 on degraded soil of mine and thermal power plant Gacko. The experiment was placed on a landfill located in Srđevići. For these tests, grass-leguminous mixtures were used: I/G (alfalfa 70%, orchard grass 30%); II/G (alfalfa 70%, orchard grass 15%, tall oat-grass 15%); III/G (red clover 50% , italian rye-grass 50%); IV/G (alfalfa 30%, tall fescue 40%, *smooth brome*15%; orchard grass 15%) and alfalfa 100%. The following parameters were monitored and analyzed during the tests: mechanical soil composition, soil chemical properties, microelement content in the soil: Zn, Mn, Fe, Cu; the content of heavy metals in the soil: Pb, Ni, Cr, Cd, Hg, As, plant species presence in the mixture, soil cover with grass, yield of green mass by cuts and years, content of microelements and heavy metals in dry vegetable mass by cuts and influence of nitrate content of individual microelements and heavy metals in soil and dry mass of plants.

Studies of the content of microelements and heavy metals in degraded soil showed that it contained Cd in significantly higher value than the permitted limit value. Other elements were within the limit values permitted for agricultural land. The content of Cd in 2011 was 5.1 mg kg^{-1} of soil, and in 2013 it was 4.5 mg kg^{-1} of soil. By analyzing the composition of the green matter in mixtures I/G, II/G and IV/G, it was found that orchard grass was predominantly present in relation to other constituents of the mixture. The III/G mixture was dominated by Italian rye-grass. The participation of other plant species in the mixture was extremely low. In the green mass of all mixtures, leguminous was represented in a small

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percentage. The largest soil cover with plants was achieved with the mixture IV/G (88.4%), which at the same time achieved the highest average yield of green mass. The determined values of the content of microelements and heavy metals in the dry mass were within the allowed limits. Nitrates influenced the reduction of Cu and Cr content in soil and Cd content in dry plant mass.

Keywords: degraded soil, recultivation, alfalfa, grass-leguminous mixtures

INTRODUCTION

Soil is the basic natural resource which, together with air, water, fauna and flora makes an ecosystem. Changes in the constituent parts of the ecosystem are reflected on the system as a whole. Soil is a resource that is difficult to regenerate. It can be used in many ways. In principle, two basic groups are distinguished: technical and ecological. From the aspect of technical function, the soil can be used for various purposes. The use of land for the purpose of exploitation of raw materials or for mining activities can lead to its permanent or temporary exclusion from production. Mining activities on mine surface lead to degradation and physical destruction of huge land surfaces, the deposit of large quantities of defects and the occurrence of mine waters.

In order to remedy the consequences of the activities on surface mining sites, sanitary measures such as: recultivation, phytomeliotation, decontamination, etc. are performed. The process of recultivation of degraded soil implies technical and biological recultivation. Technical recultivation aims to form the terrain and apply a layer of suitable material to create conditions for the development of vegetation. This part of the recultivation is extremely expensive. Most activities are directed towards transforming degraded soil into a state that is suitable for agricultural and forestry purposes. Biological recultivation is a long-lasting and complex process, which implies the application of a series of appropriate measures to accelerate pedological processes. The implementation of biological recultivation requires a preliminary analysis of the existing situation on the ground and the implementation of the phases, as well as subsequent monitoring of the process and permanent correction (Golubović et al., 2015). Before starting any activity it is necessary to perform chemical analyzes and determine the content of microelements and heavy metals in degraded soil. High concentration of heavy metals reduces soil quality, destroys biological balance and damages all other ecosystem functions (Belanović et al., 2012, Belanović et al., 2013).

Biological measures applied in the final stage of recultivation are also significant from the aspect of area revitalization and the establishment of natural biocenoses. In order to make the process of remediation of degraded soils faster, it is necessary to introduce large quantities of organic and mineral substances to compensate for the lack of basic nutrients. This requires significant financial investment and is often a limiting factor. Therefore, the processes of recultivation of degraded soil in Bosnia and Herzegovina on a large number of similar mines

take place slowly and mainly experimentally. In order to accelerate the transformation of mining degraded soils into a state suitable for agricultural use, the studies that were supposed to find the most favorable and economical solution for the revitalization of the area and the establishment of natural biocenoses have begun. Spontaneous renewal of vegetation on surfaces degraded by mining activity with the application of melorative measures proved unsuccessful. Spontaneous restoration of grass cover of varying covering degrees can last from 6 to 23 years (Csecserits et al., 2007; Feng et al., 2007a, b). Grass cover renewal on degraded surfaces is of great importance, because it provides the possibility of reducing the loss of grass surface's biodiversity (Plieninger & Gaertner, 2011). Renewal and putting in use the degraded soil surfaces is of great importance for the community, especially if there is a multicomponent contamination present, and if there are urban or settled areas in the proximity.

The aim of this research was to examine the possibility of reparing the chemical properties of substrate and regenerating the vegetation of degraded soil due to mining activity without adding the fertile soil layer.

MATERIAL AND METHOD

Field research was carried out on the land of the mine and thermal power plant in Gacko from 2011-2013. Analysis of samples of soil and plant material took place in the period 2011-2014 in the laboratories of the Agricultural Institute of Republic of Srpska, Banja Luka. Two-factorial experiment (factor A - years, and factor B - type/mixture) was set in four repetitions at the location Srđevići (949 m. altitude above sea level). The surface of the experiment was 1.240 m^2. For those tests, grass-leguminous mixtures were used: I/G alfalfa 70%, orchard grass 30%; II/G alfalfa 70%, orchard grass 15%, tall oat-grass 15%; III/G red clover 50, Italian rye-grass 50%; IV/G alfalfa 30%, tall fescue 40%, smooth brome 15%, orchard grass 15% and alfalfa 100%. The following seeds were used for sowing: alfalfa in pure culture (100%) 30 kg ha^{-1} of seed; I/G 32 kg ha^{-1}; II/G 40 kg ha^{-1}; III/G 37.5 kg ha^{-1} and IV/G 43.5 kg ha^{-1}.

Before the start of any activity on the experimental surface, the degraded soils were rigged at a depth of 60 cm and fencing was done. After that, soil samples for physical chemical analyzes were taken. On the rigid degraded soil before plowing it was dispersed 30 kg of N, 90 kg P_2O_5 and 40 kg K_2O. The plowing was done at a depth of 20-25 cm, and then 30 kg of N, 90 kg of P_2O_5 and 40 kg of K_2O were dispersed again. The preparation of land for sowing was done by a rotary harrow. The land is aligned with a light harrow. The stones on the test parcel were manually removed. The sowing experiment was carried out in September 2011. After sowing, the entire sown surface was powell with a smooth roller.

Fertilization was done with nitrogen fertilizer KAN 27%N. During the vegetation in the first cut we used 54 kg ha^{-1} N, 40.5 kg ha^{-1} N in the second cut and 27 kg ha^{-1} N in the third cut. The first topdressing was done after the

vegetation in the first cut, and the second and third was applied fifteen days after mowing the first and second cut.

The following parameters were monitored and analyzed during the test: mechanical soil composition, soil chemical properties, microelement content: Zn, Mn, Fe, Cu; the content of heavy metals in the soil: Pb, Ni, Cr, Cd, Hg, As, the presence of plant species in the mixture, grass cover on soil, yield of green mass by cuts and years, content of microelements and heavy metals in dry vegetable mass by cuts and influence of the nitrate content on individual microelements and heavy metals in soil and dry mass of plants.

Taking samples on degraded land was carried out before the establishment of the crops in September 2011 and after the removal of III grass leguminous mixture in 2013. The samples were taken at a depth of 0-25 cm. Analyzes of the samples were done in the laboratory for agro-chemistry Agricultural Institute of Republic of Srpska in Banja Luka.

- The active reaction (pH in H_2O) and the potential soil reaction (pH in KCl) were determined by combined electrode on a pH-meter electrometrically.
- The amount of humus was determined by the colorimetric method in a wet burned sample.
- The amount of physiologically active P and K was determined by the ammonium-lactate (AL) method, with the reading of the P concentration on the spectrophotometer and K on the plasma photometer.

Sampling of degraded soil for analysis of chemical properties and total content of microelements and heavy metals was done at a depth of 0-25 cm.

Methods of fractionation by means of a sieve series (for skeleton and larger fractions) and pipette method (sedimentation in calm water) were used to determine the mechanical composition of the soil.

The yield of green mass was obtained by mowing four times per 5 m^2 of plant mass of all mixtures and alfalfa. For the analysis of the chemical composition of dry matter, an average sample of each repetition was taken immediately after mowing. Taken samples of green mass were naturally dried.

During the examination, the composition of the green plant mass was analyzed. The plant mass of grass-leguminous mixtures was separated into components regarding mixtures and other plant species that were not sown. During the survey, the land cover by the crop was determined, i.e. the density of crops. Also, in the dry mass of the first cuts in 2012 and 2013, the content of microelements and heavy metals was examined, and the influence of nitrates on their content was also monitored. Coverage results and yield of green matter were analyzed by variance analysis (ANOVA), and the significance of differences between mean values was determined by LSD-test.

RESULTS AND DISCUSSION

Mechanical composition. The percentage distribution of skeletal fractions of different dimensions after analysis of the skeletal base of degraded soil is shown in Table 1. Based on skeletal fractions, the soil is classified as skeletal

medium stony. The skeleton was 62.2% in relation to total porosity. The stone fractions, separated on sieves with an aperture of 2-20 cm, were represented by 68.3%. This fraction consists of 61.0% of the particles sizing from 20 to 5 cm and 7.3% of the particles sizing 5-2 cm. The gravel fraction was separated on sieves with openings of 0.2-2.0 cm, and it was represented with 31.7%. This fraction consisted of 13.8% of the particles sizing 1-2 cm, 8.9% of the particle sizing 0.5-1 cm and 9% of fine gravel particles sizing 0.2-0.5 cm.

Table 1. Analysis of the skeletal base - degraded land according to Gračanin (1947)

Depth (cm)	Skeleton in relation to total porosity, %	Skeletal fractions (cm)								Class
		Stones, %				Gravel, %				
		>20	20-5	5-2	Total	2-1	1-0.5	0.5-0.2	Total	Skeletal, medium stony
0 – 60	62.2	0	61.0	7.3	68.3	13.8	8.9	9.0	31.7	

Chemical properties of the degraded soil According to the results of chemical analyzes of the layer of degraded soil, alkaline reaction (pH in H_2O 8.4) was found at the depth of 0-25 cm. There was no humus in the analyzed layer.

Table 2. Chemical properties of the degraded land

Year	Depth (cm)	pH		Humus (%)	N (%)	$CaCO_3$ (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
		H_2O	KCl					
2011	0-25	8.4	8.3	-	0.01	98.3	1.1	1.3

The content of easily accessible phosphorus was very low (1.1 mg/100 g of soil). The provision of potassium was also low (1.3 mg/100 g of soil). Based on the content of CaCO3 (98.3%), the degraded soil was classified into highly carbonate soils.

The content of microelements and heavy metals in degraded soil The content of microelements and heavy metals in a layer of 0-25 cm of degraded soil is shown in Table 3.

Table 3. Total content of microelements and heavy metals in degraded soil

Year	Depth (cm)	The content of elements (mg kg^{-1})									
		Mn	Fe	Zn	Cu	Pb	Cd	Ni	Cr	As	Hg
2011	0-25	0.01	0.1	5.4	5.7	7.3	5.1	21	30	0.4	<0.1
2013	0-25	0.01	0.1	6.1	5.1	6.8	4.5	22	27	0.6	<0.1
Permitted values in agricultural land*		1	4	200	120	150	2	75	100	20	1.5

* Rulebook (2016)

The total content of the microelements Mn and Fe was within the allowed limits. The content of the investigated heavy metals, except Cd, was below the permitted limit value in agricultural land. The content of Cd in the degraded soil varied from 4.5 mg kg^{-1} (2013) to 5.1 mg kg^{-1} (2011) and was higher than allowed values in both years of testing.

The representation of plant species in the mixture In order to gain insight into the representation of individual components in grass-leguminous mixtures, analyzes of the composition of the planted mass in the first cuts in 2012 and 2013 were performed during the experiments. The results of the analysis of the composition of herbaceous grass-leguminous mixtures of first cut in 2012 are shown in Figure 1-4.

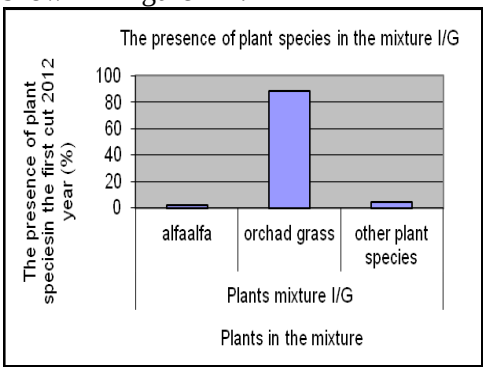

Fig. 1. The presence of plant species in the mixture I/G

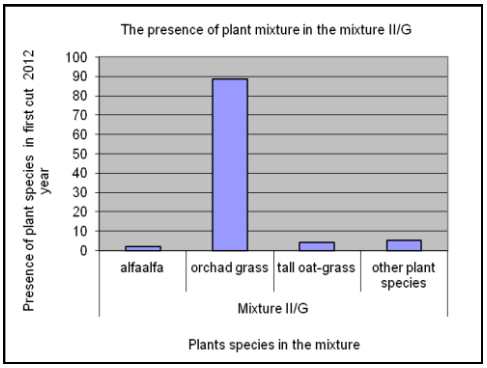

Fig. 2. The presence of plant species in the mixture II/G

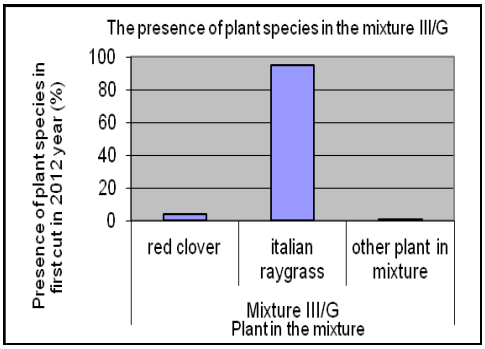

Fig. 3. The presence of plant species in the mixture III/G

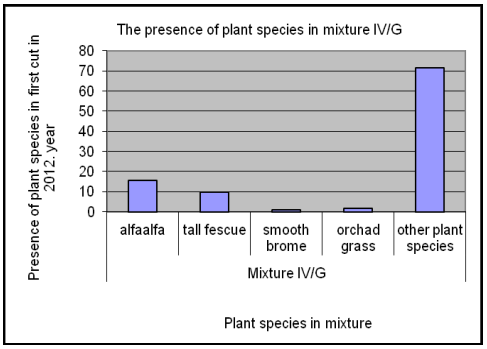

Fig. 4. The presence of plant species in the mixture IV/G

Analyzing I cut of the grass-leguminous green mass of mixtures in 2012, a slight share of other plant species in I/G, II/G and III/G (1.1% to 5.2%) was found. The participation of other plant species in IV/G mixture was dominant (71.5%). Other plant species in the mixtures were represented by 28.5%. In I/G and II/G mixtures the dominant plant was Orchard grass, while the participation of the other components in the mixture was insignificant (Charts 1 and 2). In green mass of III/G mixture, the most common plant species was the Italian rye-grass.

The results of the analysis of herbaceous mass in grass-leguminous mixtures of I cut in 2013 are shown in Figure 5-8.

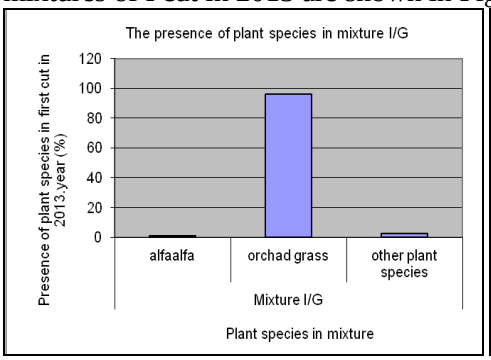

Fig. 5. The presence of plant species in the mixture I/G

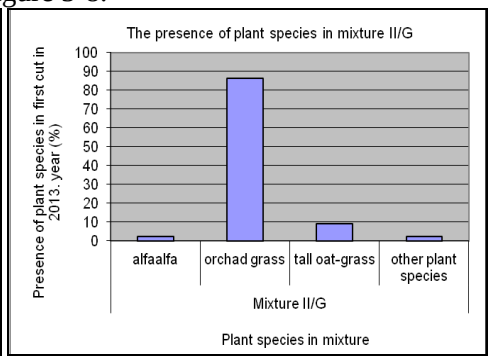

Fig. 6. The presence of plant species in the mixture II/G

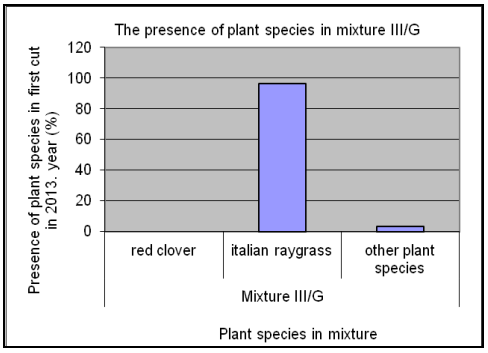

Fig. 7. The presence of plant species in the mixture III/G

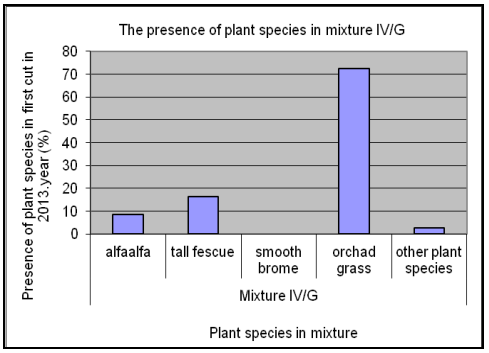

Fig. 8. The presence of plant species in the mixture IV/G

During the second year of experiment, the green mass of I cut was dominated by grass in all mixtures. Orchard grass was the most common in herbal mass in I/G, II/G and IV/G, while Italian rye-grass was dominant in III/G. The participation of other components in the mentioned mixtures was exceptionally low. Legumes were represented in a small percentage. On all types of grasslands and at all test sites, the average share of grasses in grass biomass was 61-68%, the prevalence of legumes was 4-7%, and the share of plants from other families was 29-33% (Dubljević et al., 2020).

Coverage of degraded soil with plant cover. The results of examining the coverage of degraded soil with plant cover are shown in the Table 4.

The analysis of variance determined the existence of statistically significant differences between examined treatments. The type/mixture (factor B) and interaction of species/mixtures x years (AxB) had a very significant impact on soil cover. The impact of the year on the cover (the plant cover density) of the degraded soil was not significant.

During the first study year, the best coverage of degraded soil was achieved with the mixture III/G (89.8%). In 2013, the highest coverage was found in mixture II/G (94.1%). From the results shown in Table 5, it can be seen that the coverage of the degraded soil by plant cover in the second year of testing was

higher in alfalfa and all mixtures, except for the mixture III/G. At the III/G mixture, a decrease in the coverage of the surface of the degraded soil by plant cover was found for 43%. The highest increase in the coverage of degraded soil by plant cover was in the second testing year with the mixture II/G (12.7%). During these trials, the highest average land cover was achieved with a mixture of IV/G (88.4%). The mixture III/G during these tests had the weakest coverage (68.3%).

Table 4. Degraded soil coverage with plant cover (%)

Type/mixture	Soil cover per year, (%)		Average	Increase/decrease of soil cover 2012-2013, %
	2012	2013		
Alfalfa	81.9	91.1	86.5	+10.0
Mixture I/G	83.4	92.9	88.2	+9.5
Mixture II/G	81.4	94.1	87.7	+12.7
Mixture III/G	89.8	46.8	68.3	-43.0
Mixture IV/G	83.1	93.8	88.4	+10.7
Factors	A	B	AB	
F-calculated	244.056**	0.222ns	462.748**	
LSD 0.05 0.01	 0.99 1.31	 1.57 2.07	 2.21 2.93	

ns – non-significant; ** highly significant

The yield of green mass of alfalfa and grass-leguminous mixtures. The achieved biannual green mass yield of alfalfa and grass-leguminous mixtures grown on soil degraded by mining activity are shown in Table 5 and Figure 9 and 10. The method of variance analysis revealed that the differences between yields of tested species are statistically highly significant. Also, during these testing, highly significant effects of the year and interactions of types/mixtures x years (AxB), was established.

Tab. 5. Green mass yields of alfalfa and grass-leguminous mixtures on soil degraded by mining activity

Type/mixture		Green mass yield (t ha^{-1})		$\bar{X}$
		2012	2013	
Alfalfa		0.5	21.0	10.8
Mixture I/G		0.4	23.3	11.8
Mixture II/G		0.3	17.6	8.9
Mixture III/G		4.5	21.2	12.8
Mixture IV/G		0.4	25.8	13.1
Basic factors		A	B	AB
F - calculated		236.17**	43221.37**	276.26**
LSD	0.05 0.01	0.2 0.3	0.3 0.4	0.4 0.6

** highly significant

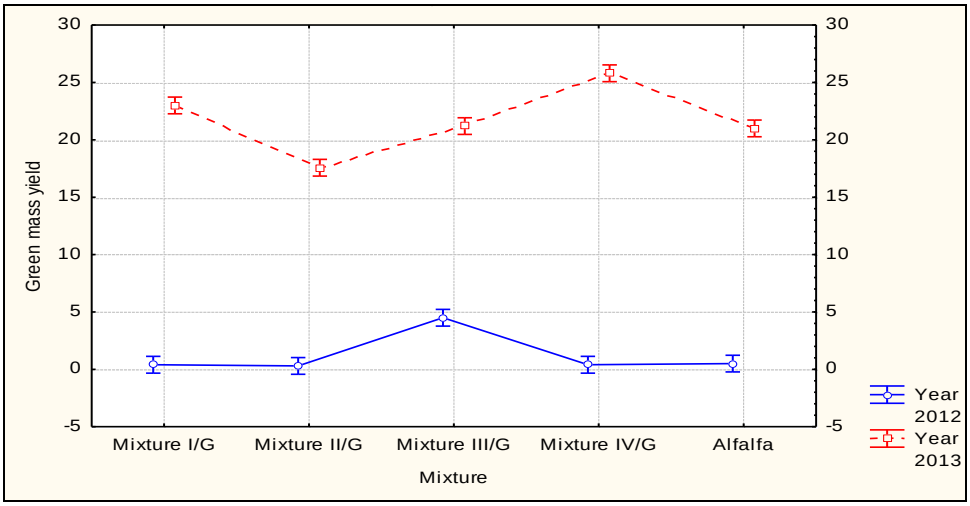

Fig. 9. The effect of the year and the mixture on the yield of green mass

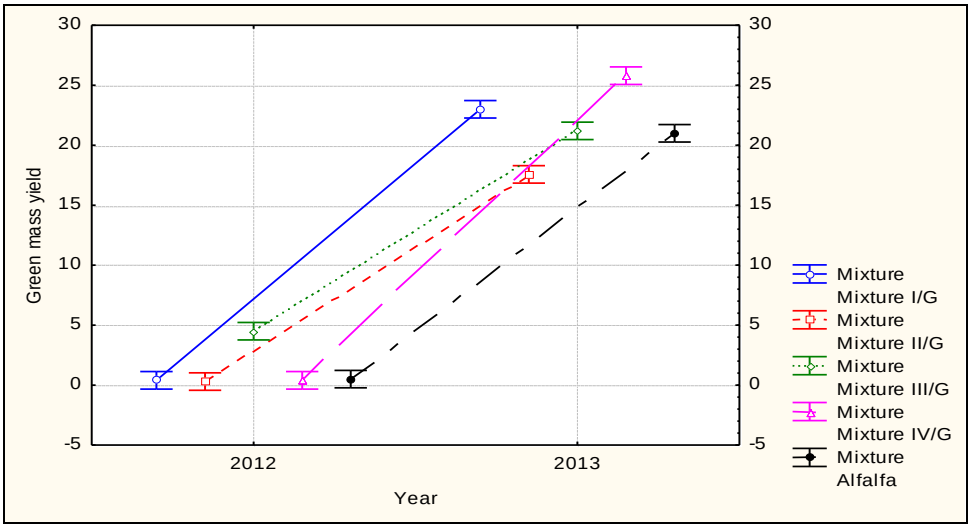

Fig. 10. The effect of the mixture on the yield of green mass

Low yields of green mass were achieved in the first year of the survey. During 2012, regarding the yield of green mass, the mixture of III/G (4.5 t ha^{-1}) was prominent. In the following year significantly higher yields were achieved for all sown types/mixtures. The highest growth of yield of green mass in the course of these tests was achieved with a mixture of IV/G (25.8 t ha^{-1}).

The content of microelements and heavy metals in plant mass. After mowing the first cut, the content of microelements and heavy metals of iron, manganese, zinc, copper, lead, cadmium, nickel, arsenic and mercury was tested in dry plant mass. Table 6 shows the results of the analysis of dry plant mass and of the first cut in 2012.

Table 6. The content of microelements and heavy metals in dry plant mass in 2012 per cuts

Mixture	Cut	Heavy metals and microelements in dry matter, mg kg^{-1}									
		Fe	Mn	Cu	Zn	Pb	Cd	Ni	Cr	Hg	As
Mixture I/G	I	138	27	2	21	8	0.25	3.1	< 0,1	<0.05	<0.1
Mixture II/G	I	133	21	3	23	13	0.26	5.0	< 0,1	<0.05	<0.1
Mixture III/G	I	45	13	2	23	2	0.25	4.8	< 0,1	<0.05	<0.1
	II	159	28	13	43	2,1	0.25	1.4	<0,1	<0.05	<0.1
Mixture IV/G	I	263	47	6	19	7	0.34	5.1	< 0,1	<0.05	<0.1

Table 7. The content of microelements and heavy metals in dry plant mass in 2013 per cuts

Type /mixture	Cut	Heavy metals and microelements in dry mass (mg kg^{-1})									
		Fe	Mn	Cu	Zn	Pb	Cd	Ni	Cr	Hg	As
Alfalfa	I	132	17	3	23	2.5	0.30	2.3	< 0.1	<0.05	<0.1
	II	76	22	9	18	2.6	0.14	4.5	< 0.1	<0.05	<0.1
	III	93	16	9.6	20	1.0	0.09	1.8	< 0.1	<0.05	<0.1
Mixture I/G	I	105	21	6	22	2.3	0.22	1.1	< 0.1	<0.05	<0.1
	II	117	33	11	19	2.4	0.14	2.6	< 0.1	<0.05	<0.1
	III	68	19	9.5	17	1.3	0.12	1.9	< 0.1	<0.05	<0.1
Mixture II/G	I	96	17	6	21	2.1	0.29	2.9	< 0.1	<0.05	<0.1
	II	125	26	8	21	3.1	0.21	1.5	< 0.1	<0.05	<0.1
	III	68	17	3,2	21	1.4	0.07	1.2	< 0.1	<0.05	<0.1
Mixture III/G	I	125	16	3	20	2.5	0.42	1.1	< 0.1	<0.05	<0.1
	II	130	24	7	24	3.6	0.24	1.5	< 0.1	<0.05	<0.1
	III	77	14	7.1	22	1.1	0.09	1.3	< 0.1	<0.05	<0.1
Mixture IV/G	I	105	20	9	19	2.3	0.30	3.8	< 0.1	<0.05	<0.1
	II	68	28	11	20	1.4	0.20	2.7	< 0.1	<0.05	<0.1
	III	96	25	14.5	20	1.0	0.08	1.0	< 0.1	<0.05	<0.1

According to the study results of dry plant mass per cuts in 2012, the content of microelements and heavy metals was within the allowed limits. During that year, only the mixture III/G had two cuts. In the plant material of the second cut, a higher content of Fe, Mn, Cu, Zn and Pb was determined in relation to the content of the same elements in the first cut. The content of Cd remained the same as in the first cut.

The study results of the content of microelements and heavy metals in dry plant mass of the first, second and third cut in 2013 are shown in the Table 7.

The obtained values of the content of microelements and heavy metals in the dry plant mass of all cuts were within the allowed limits. It is interesting that the content of Mn, Cu and Pb in all mixtures/types was higher in the second cut compared to the first one. The content of Cd was reducing in plant weight in each subsequent cut in all mixtures/alfalfa.

The structure of the soil is of great importance for plant production. If the soil structure is favorable, it can, to a certain extent, affect the unfavorable mechanical composition. This is most often manifested in such a way that a favorable soil structure influences the provision of a favorable aquatic-air regime in the soil and provides conditions for the normal growth and development of the root of the plant (Racz, 1994).

Contamination of agricultural lands with heavy metals presents risk and danger for humans (Xu et al., 2017). Arenas-Lago et al. (2014) conducted tests on the mobility of Pb, Zn and Cd in the area of mining zone of Rubiais - Lugo, Spain. Tests were conducted at nine locations. The pH values of the soil affects the bioaccesibility of metals and the process of their absorption trough the root.During the testing of the land in mining zones, the following concentrations of the tested heavy metals were: Pb 0.850-6.761 mg kg^{-1}, Zn 1.754-32.287 mg kg^{-1} and Cd 1.8-43.7 mg kg^{-1}. The above Pb values were compatible with our results, while the above values for Zn and Cd in those studies were significantly above the highest values we found during these studies. Kabata-Pendias et al. (1989) found that the average content of total Zn in the surface layers of agricultural land varied from 17 to 125 mg kg^{-1}, which was below the permissible limit value of 200 mg kg^{-1} of soil.

During the restoration of the lawns in the Hortobagi National Park in eastern Hungary, two grass mixtures suitable for alkaline soils were used: *Festuca pseudovina* (67%), *Poa angustifolia* (33%) and *Festuca rupikola* (40%), *Bromus inermis* (30%), *Poa angustifolia* (30%). The sowing norm was 25 kg ha^{-1} (Török et al., 2012). Approximate quantities of seed during the restoration of the lawn were used by Kiehl et al. 2010; Török et al. 2011a. The given quantities of the used seed were slightly lower than those used during our research. The larger quantities of seeds during the restoration of lawns on degraded surfaces were reported by Van Andel & Aronson (2006). Orchad grass can succeed on different types of soil. This plant species tolerates drought well, it is able to regenerate rapidly after mowing or grazing and has a good summer increase (Lakić et al., 2009). During these studies, orchard grass was predominantly represented in the

green mass of I/G, II/G and IV/G. The mixture III/G was dominated by the Italian ray grass, while the red clover was represented in traces. Török et al. (2011b) stated that with the sowing standard of alfalfa of 30 kg ha^{-1} in the first year the land cover of over 70% was achieved, which was in accordance with our results. According to the above-mentioned authors, the soil coverage began to decrease gradually only after the third year. During the research carried out by Li Xu & Vang (2008), the alfalfa sowing norm was 22 kg ha^{-1} of seed, and in the first year the soil coverage was about 50%.

The use of vegetative cover for soil remediation contaminated with heavy metals requires research to determine the plant species that are most suitable for this matter (Bider et al., 2007). The mentioned authors studying which plant species were the most suitable for the formation of the vegetative cover found that heavy metals such as Cd, Pb and Zn were mostly accumulate in the root, and considerably less in the above-ground part. Symanowicz et al. (2014) examined the influence of nitrates (ammonium sulphate) on the content of heavy metals Cu, Zn, Ni, Cr, Pb and Cd in the dry mass of *Galega orientalis* Lam. During the three-year trials, the abovementioned authors found that nitrates influenced the reduction of Zn, Ni and Cd content in dry herbaceous matter. It was also found that the application of nitrates decreased Cu, Zn and Cr levels in the soil. According to the results of our study, nitrates contributed to the reduction in the dry matter content of Cd in all mixtures and alfalfa during 2013. The content of Zn and Ni gradually decreased in dry weight only in certain grass-leguminous mixtures. In three tests of locations in Montenegro, the content of Ni in the country was higher than the maximum allowed amounts, while the available concentration of Ni was between 0.6-7.9 mg kg^{-1}, and its concentration in plants collected from all locations was below 10 mg kg^{-1} (Simić et al., 2019). Also, the results obtained during these trials were in line with the data of Symanowicz et al. (2014) regarding the effect of nitrate on the reduction of Cu and Cr content in the soil. Karami et al. (2011) cultivated by perennial ryegrass *Lolium perenne* L. var. Cadix after the introduction of compost and biological waste on the land of the former copper mine in Cheshire (UK), which was heavily contaminated with Pb and Cu. Gadepalle et al. (2008) questioned the acceptance of As from contaminated soil by plants after the introduction of compost, zeolite and iron oxide into the soil. The results of the study showed that the plants (*Lolium perenne*) took less than 0.01% of the total As content in the soil. In the dry mass of the Italian raygrass, which was grown on low acidity soil (pH 6), the first cut had a content of Cd 0.79 mg kg^{-1}, Pb 7.98 mg kg^{-1}, Zn 91.0 mg kg^{-1}. The dry mass of the second cut had a content of Cd 0.74 mg kg^{-1}, Pb 7.85 mg kg^{-1}, Zn 93.5 mg kg^{-1} (Kwiatkowska-Malina & Maciejewska, 2013). The values indicated by the mentioned authors were significantly higher than the values determined during these tests in the dry weight of the Italian ray grass and red clover.

The concentration of heavy metals in plants indicates the degree of contamination, but also the ability of different plant species to accumulate metals from the soil. If plants are planted on soil with high content of heavy metals they

will absorb and accumulate them in productive organs (fruits or aboveground biomass). The use of such plants for the feeding of domestic animals causes them to be contaminated with heavy metals as well. Meat, milk and eggs of domestic animals fed with such vegetable food will be of low quality and will have a high content of heavy metals (Mitkova et al., 2005).

CONCLUSION

Degraded soil contained significantly higher concentration of cadmium (Cd) than maximum allowed border values for agricultural soil while other tested elements were within the allowed border values. In the soli degraded by mining activity, the content of cadmium (Cd) gradually decreased, but it's content was still above maximum values allowed for agricultural soil.

The analysis of green mass content in mixtures I/G, II/G, III/g and IV/G established that orchard grass (*Dactylis glomerata* L) was dominantly represented compared to other components in the mixtures. The *Italian raygrass* was dominant in the III/G mixture. The participation of other species in the mixtures was extremely low. Legouminous plants were represented in a small percentage in every mixture's green mass.

The biggest plant cover of the soil was achieved with the IV/G mixture (88.4%), which also had the biggest average of green mass yield. The determined values of heavy metals and microelements contents were in the allowed limits.

The nitrates affected the decrease in copper (Cu) and chrome (Cr) content in the soil and cadmium (Cd) content in the plant's dry mass.

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Gordan S. KARAMAN [1]

BIODIVERSITY OF SUBTERRANEAN WATERS: REDESCRIPTION OF POORLY KNOWN *NIPHARGUS JULIUS* STOCH 1997 (CRUSTACEA: AMPHIPODA: NIPHARGIDAE) FROM ITALY (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 314)

SUMMARY

The subterranean species *Niphargus julius* Stoch 1997 (Crustacea: Amphipoda: Niphargidae), known from Prealpi Giulie (Friuli-Venetia Giulia region in Italy), described by Stanko Karaman (1954) as *Niphargus stygius d`anconae*, ssp. n. from cave "Grotta near Villanuova", was later renamed as *Niphargus julius*, new name, by F. Stoch (1997), because name "**danconae"** was already used for another species, *Niphargus danconai* Benedetti 1942 described from Verona (Italy). *Nipharus julius* was collected in various subterranean waters, mainly caves of Prealpi Giulie region, but after the short original description of this taxon of S. Karaman (1954), this species was never described and figured more in detail. This species is here described and figured based of material from type-locality. The some relations of this species regarding other similar species from NE Italy are discussed.

Keywords: taxonomy, redescription, *Niphargus julius*, Amphipoda, Niphargidae, Italy

INTRODUCTION

The subterranean waters of Europe, including Italy, are with very rich subterranean fauna only partially known and discovered. As these waters are today under pressure of overexploitation, pollution and other anthropogenic activity, the subterranean fauna existing in these waters, including Amphipoda, is in danger to be partially or completely destroyed before its discovery and investigation. As the fauna of Amphipoda in the subterranean waters of Italy is very rich and highly endemic, one detailed research of these animals seems to be very urgent.

In the previous period, when remarkably less number of species of *Niphargus* have been known, a much smaller number of taxonomical characters were used in description of new species, descriptions were relatively short and

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poorly figured. Along with the increase in the number of discovered species of *Niphargus*, there is an urgent need for more detailed description and figures of each species to facilitate the delimitation among similar taxa. For this reason we selected the subterranean species *Niphargus julius* Stoch 1997 (Crustacea: Amphipoda: Niphargidae), only partially known from Friuli-Venetia Giulia region in Italy, to redescribe it and draw more in detail, and by this way to make easier recognition of this species regarding several other similar species of N. Italy and Slovenia.

This species was described as *Niphargus stygius danconae*, ssp. n., by Stanko Karaman (1954) from caves near Villanuova, but this name was nom. preocc. Name "danconae" was already used for another species, *Niphargus danconai* Benedetti 1942 described from Verona (Italy). Stoch (1997) put a new name for it, *Niphargus julius*, new nom., without any additional description of this taxon.

MATERIAL AND METHODS

The studied material was preserved in the 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All body-parts were temporarily submersed in the mixture of glycerin and water for study and drawing. The body-length of examined specimens were measured by tracing individual's mid-trunk lengths (from tip of head to end of telson) using camera lucida. All illustrations were inked manually.

After study, all appendages were transferred to Liquid of Faure on permanent slides. The advantage of use of Liquid of Faure is that it is possible to remove again all dissected body-parts from Liquid of Faure by water and study it again from various positions under the microscope. After the study, these body-parts can be put on slides in Liquid of Faure again and covered by thin cover glass making definitive microscopic slides.

Some morphological terminology and setal formulae follow G. Karaman's terminology (Karaman, G., 1969; 2012) regarding the distal mandibular palpus article [A = A-setae on outer face; B = B-setae on inner face; C = additional setae on outer face; D = lateral marginal D-setae; E = distal long E-setae], and propodus of gnathopods 1 and 2 [S = corner S-spine; L = lateral slender serrate L-spines; M = facial corner M-setae; R = subcorner R-spine on inner face]. Terms "setae" and "spines" are used based on its shape, not origin.

Our studies are based on the morphological, ecological and zoogeographical investigations.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS JULIUS STOCH 1997

Figures 1-7

Niphargus (Stygoniphargus) stygius D' Anconae n. ssp., S. Karaman, 1954: 164, figs. 9-11;

Niphargus stygius danconai G. Karaman & Ruffo, 1986: 532; Stoch, 1993: 180; Stoch, 1996: 85.
Niphargus stygius G. Karaman, 1986: 215;
Niphargus stygius (part.) G. Karaman, 1993: 263;
Niphargus julius, n. nov., Stoch, F., 1997: 96, 98., Stoch, F. 2008: 110, 1 photo.

MATERIAL EXAMINED: ITALY:
-346= Puint de Pratis, Friuli, Italy, 28.4.1929, one exp. (leg. Strasser) and one slide;
-347= cave near Villanuova, Friuli, Italy, 12.8.1928, 2 exp. (leg. Strasser) and slide of holotypus;
S-6193= Grotta Nuova di Villanova Cave, Tarcento, NE Italy, 11. 11.1949, 4 exp. (leg. Busolini).

DIAGNOSIS

Metasomal articles with 8-10 dorsoposterior marginal short setae each. Epimeral plate 3 angular in males and females. Urosomal segment 1 at both dorsolateral sides with one seta in male and female, urosomal segment 2 with 2 spines. Mandibular palpus article 3 with elevated number of A and B-setae. Maxilla 1 inner plate with 2 setae, outer plate with 7 spines (6 spines with one lateral tooth only). Maxilliped inner plate with 3-4 distal spines, palpus with 2-3 setae at inner margin near basis of the nail. Coxae relatively short, coxa 4 unlobed posteriorly. Propodus of gnathopods 1-2 trapezoid, with inclined palm and elevated number of transverse rows of setae at posterior margin of propodus; Basipodit (article 2) of pereopods 5-7 dilated but without ventroposterior lobe; peduncle of pleopods 1-3 scarcely setose and with 2 retinacula each.

Uropod 1 peduncle with dorsointernal row of setae (except distal spine) and dorsoexternal row of spines, inner ramus only poorly longer than outer one in males and females. Uropod 3 long in males and females, but distal article of outer ramus shorter in females; telson strongly spinose, nearly as long as broad or poorly broader than long, provided with group of distal and facial spines, as well as with single lateral spines.

DESCRIPTION. [S-6193, Cave Nuova di Villanova]:

MALE 17.1 mm: Body strong, mesosomal segments dorsally smooth, metasomal segments 1-3 with 8-10 short dorsoposterior marginal setae (fig. 1G). Urosomal segment 1 with one dorsolateral seta on each side; urosomal segment 2 on each dorsolateral side with 2 spines and 2 setae, urosomal segment 3 naked (fig. 3A). Urosomal segment 1 with one weak spine at ventroposterior corner near basis of uropod 1-peduncle (fig. 3A).

Epimeral plate 1 with poorly marked ventroposterior corner and convex posterior margin provided with up to 8 short setae (fig. 1G), ventral margin slightly concave in the middle.

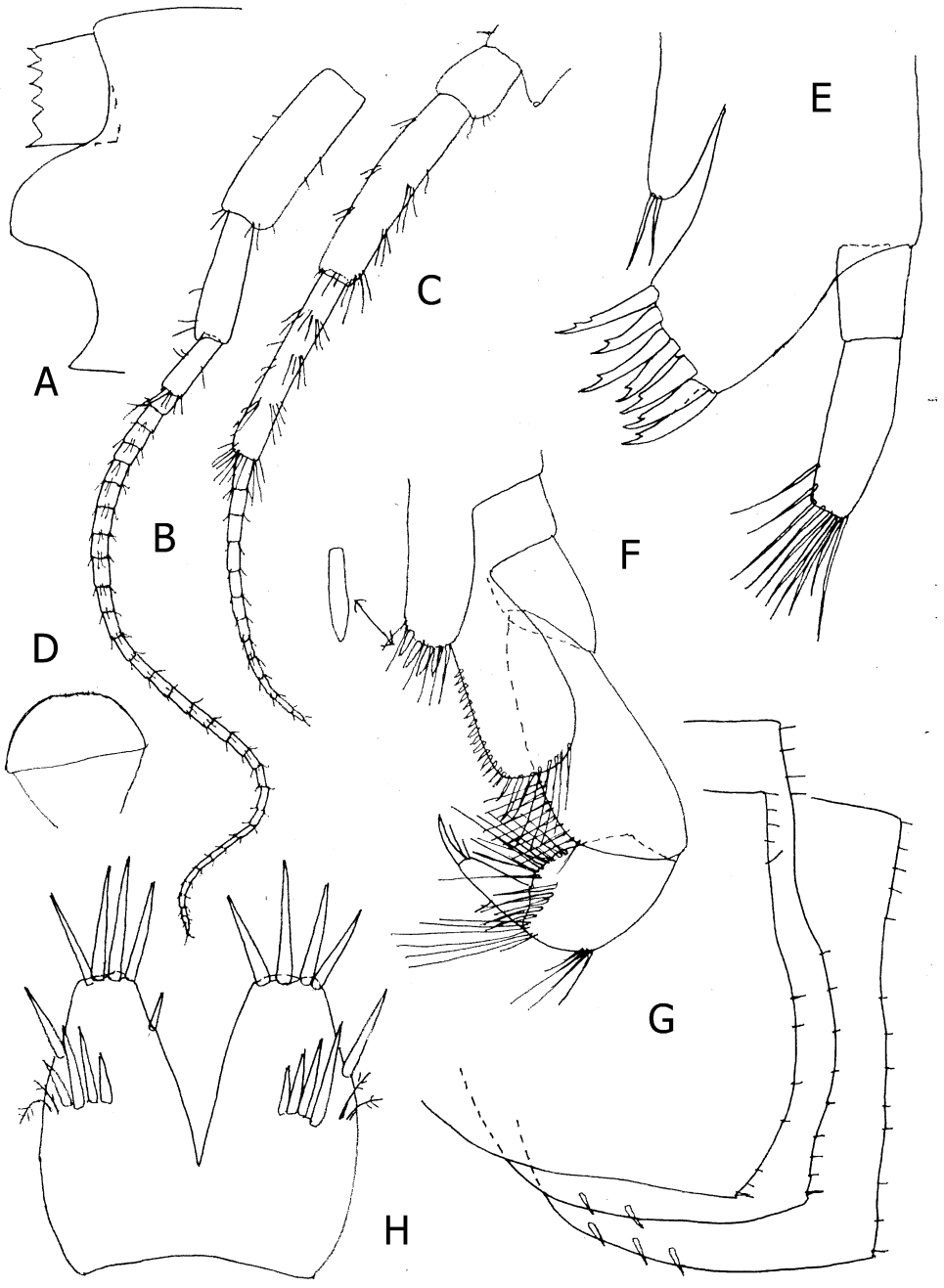

Fig. 1. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Male 17.1 mm: A= head; B= antenna 1; C= antenna 2; D= labrum; E= maxilla 1; F= maxilliped; G= epimeral plates 1-3; H= telson.

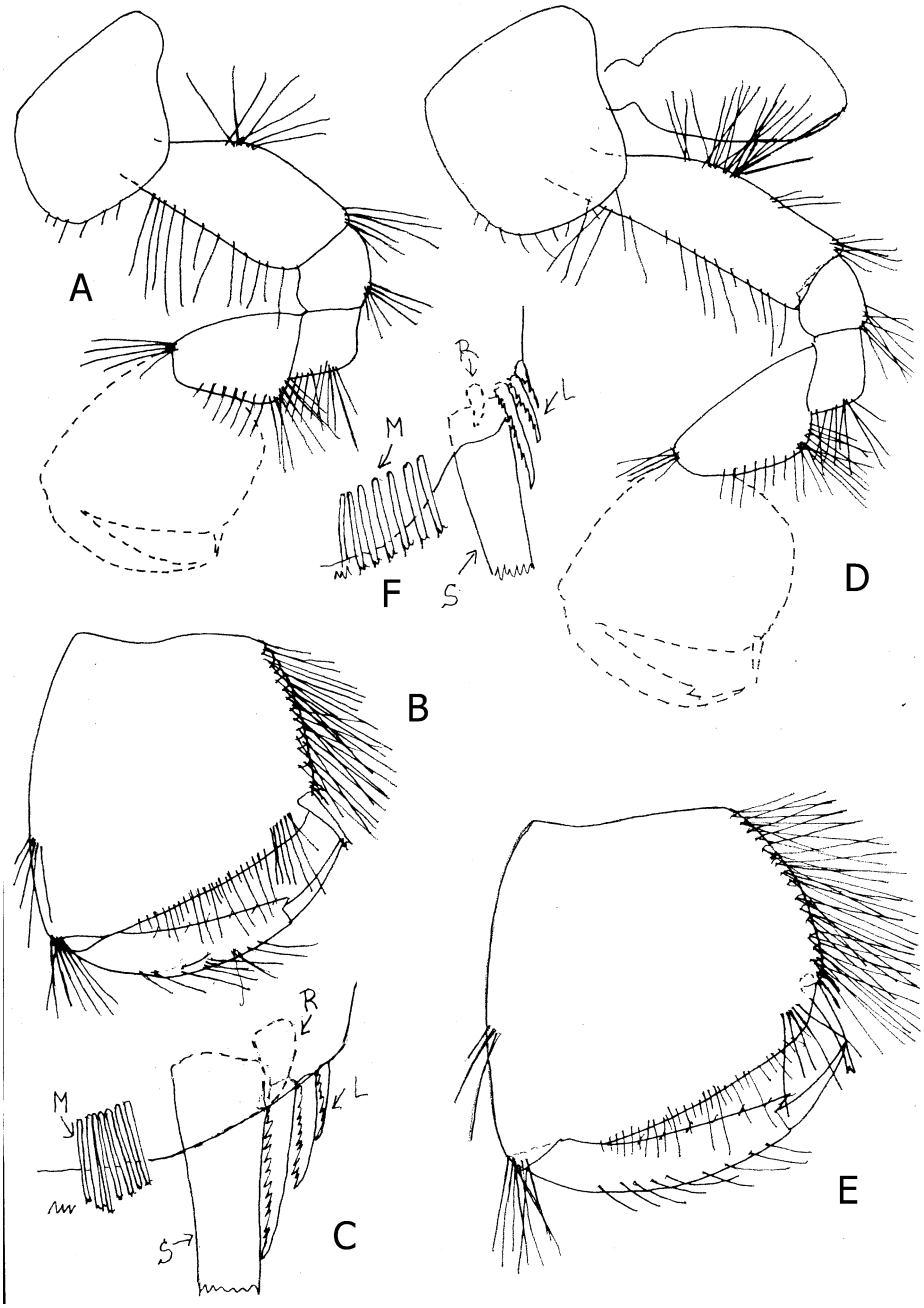

Fig. 2. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Male 17.1 mm: A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus [S= corner spine; L= lateral spines; R= subcorner spine; M= facial M-setae]; D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2 propodus [S= corner spine; L= lateral spines; R= subcorner spine; M= facial M-setae];

Epimeral plate 2 angular, with marked ventroposterior corner and slightly sinusoid posterior margin bearing nearly 9 short setae, ventral margin convex, bearing 2 submarginal spines.

Epimeral plate 3 distinctly angular, with nearly vertical posterior margin bearing nearly 8 short setae; ventral margin convex, with 3 submarginal spines. Ventroposterior corner of all three epimeral plates with stronger corner seta (not spine) (fig. 1G).

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

Antenna 1 reaching nearly half of body, peduncular articles 1-3 progressively shorter (ratio: 49:35:19), scarcely setose (fig. 2); main flagellum consisting of 32 articles (some of them with one aesthetasc); accessory flagellum short, 2-articulated, reaching nearly half of last peduncular article (fig. 1B).

Antenna 2 relatively slender; peduncular article 4 slightly longer than article 5 (ratio: 59:55), both articles bearing several bunches of short setae, mostly shorter than diameter of article itself (fig. 3); flagellum slender, longer than last peduncular article and consisting of 12 articles; conus excretorius short (fig. 1C).

Mouthparts well developed. Labrum broader than long, with entire distal margin (fig. 1D). Labium broader than long, inner lobes well developed, outer lobes entire (fig. 5A).

Mandibles with triturative molar. Left mandible: incisor with 5 teeth, lacinia mobilis with 4 strong teeth, and 6 rakers. Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, with several teeth, and 7 rakers. Palpus 3-articulate: first article naked, second article with 16 setae; third article subfalciform, rather longer than article 2 (ratio: 75:67), provided with nearly 5-6 E-setae, 32 D-setae, on outer face with 2 groups of A-setae (10+2) (fig. 3B), on inner face with 4 groups of setae (5-4-4-2) (fig. 3C).

Maxilla 1: inner plate short, with 2 setae; outer plate with 7 distal spines (6 spines with one lateral tooth, one spine with 2 lateral teeth); palpus 2-articulate, distal article not reaching distal tip of spines and provided with 10 setae (fig. 1E).

Maxilla 2: both plates longer than broad, inner plate rather smaller than outer one, both with distomarginal setae only (fig. 3D).

Maxilliped: left inner plate with 4 distal spines mixed with several setae; right inner plate with 3 distal spines mixed with several setae; outer plate reaching up to half of second palpus article and provided with nearly 15-16 marginal pointed spines and distal setae (fig. 1F); palpus 4-articulated, article 3 along outer margin with one median and one distal group of setae; article 4 at outer margin with one median seta, along inner margin with 2 setae near basis of the nail (fig. 1F).

Coxae 1-4 moderately short. Coxa 1 broader than long (ratio: 50:38), with subrounded ventroanterior corner and provided with 6-7 marginal setae (fig. 2A).

Coxa 2 hardly longer than broad (ratio: 53:51, ventral margin subrounded and provided with nearly 9 marginal setae (fig. 2D).

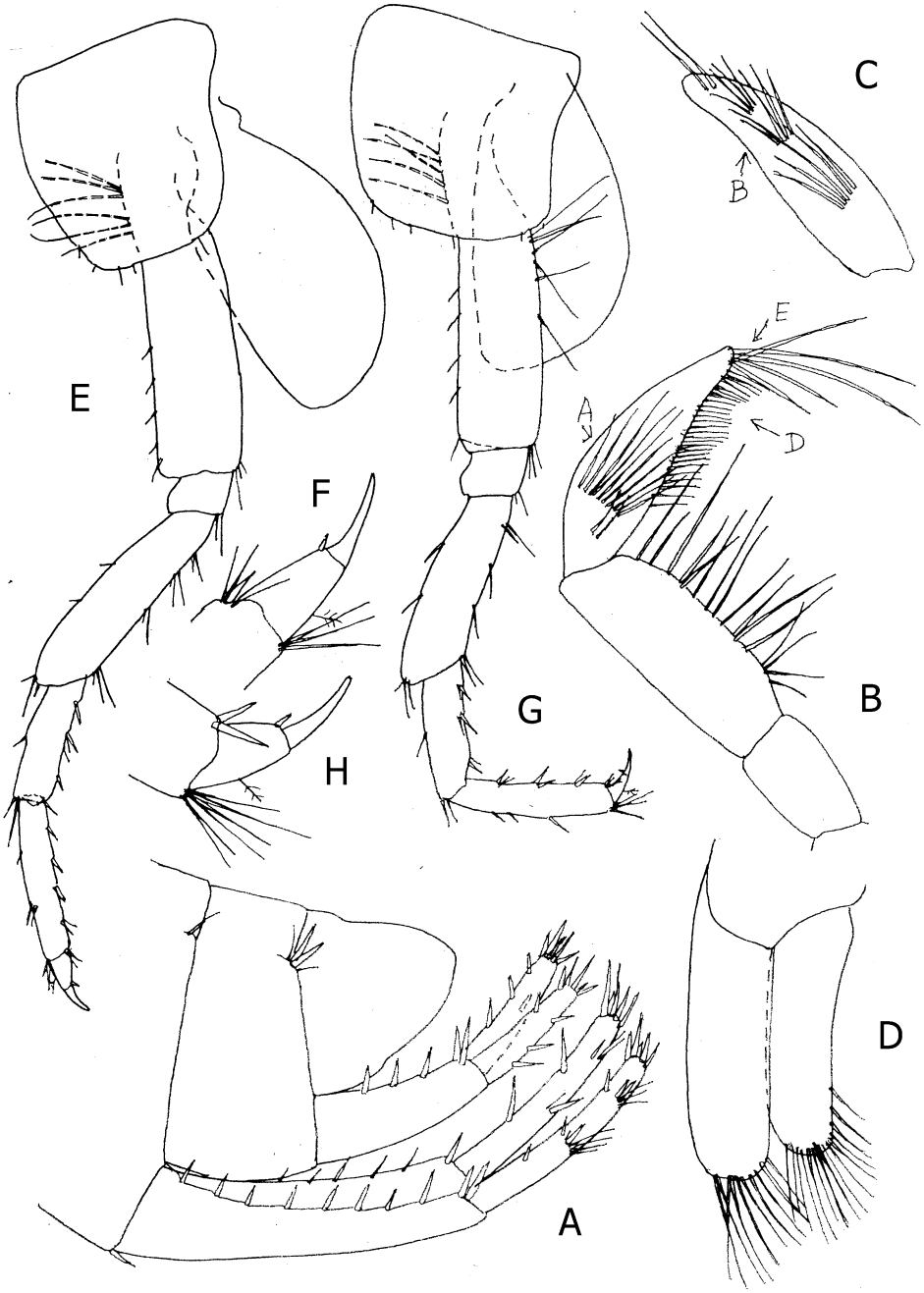

Fig. 3. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Male 17.1 mm: A= urosome with uropods 1-2; B= mandibular palpus, outer face [A= A-setae; D= D-setae; E= E-setae]; C= distal palpus article, inner face [B= B-setae]. D= maxilla 2; E-F= pereopod 3; G-H= pereopod 4.

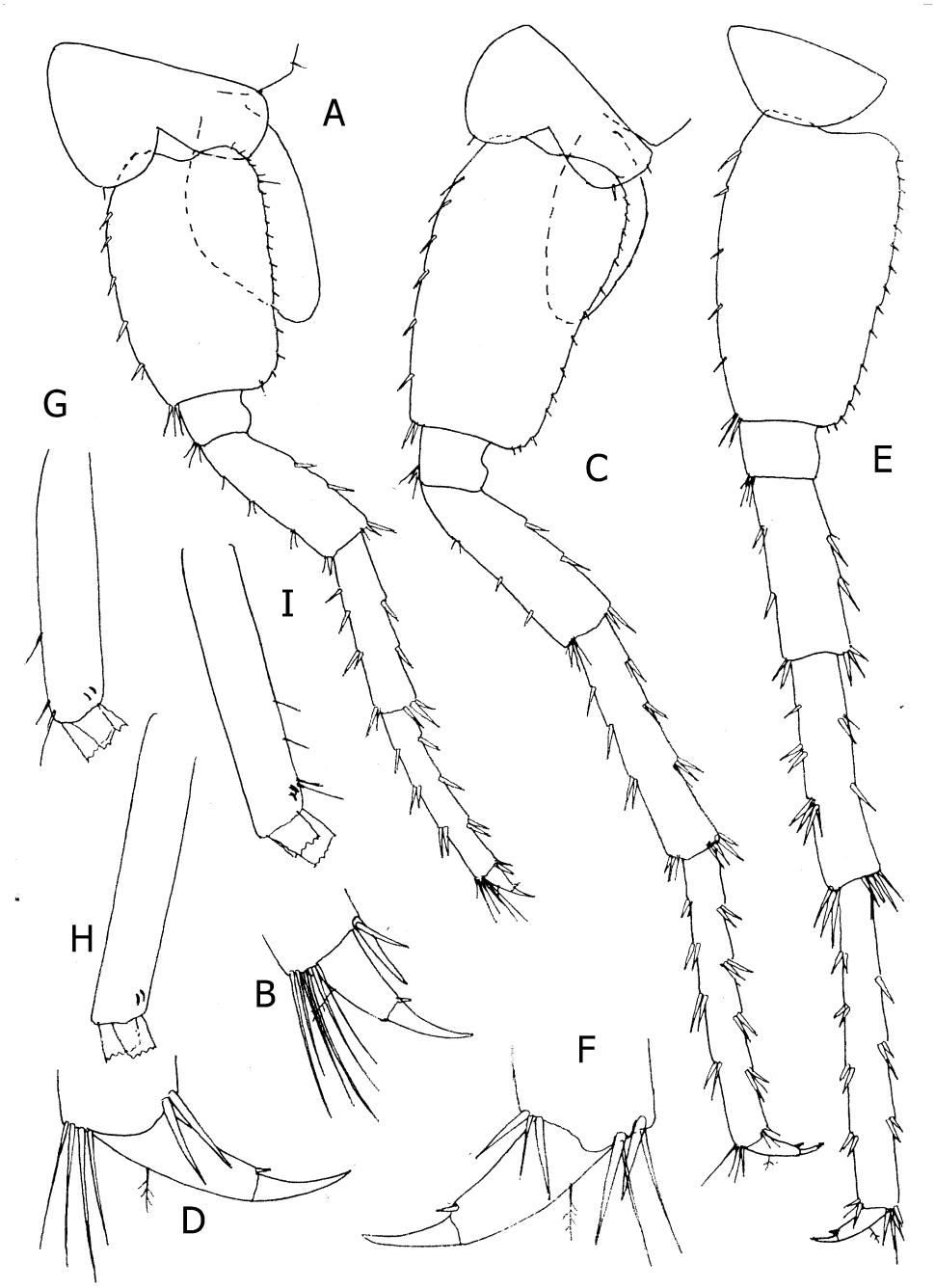

Fig. 4. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Male 17.1 mm: A-B= pereopod 5; C-D= pereopod 6; E-F= pereopod 7; G= pleopod 1 peduncle; H= pleopod 2=peduncle; I= pleopod 3 peduncle.

Coxa 3 only slightly longer than broad (ratio: 58:55) with nearly 5 marginal setae (fig. 3E). Coxa 4 slightly broader than long (ratio: 59:55), with nearly 6 marginal setae, ventroposterior lobe not developed (fig. 3G).

Coxae 5-7 relatively short. Coxa 5 bilobed, much broader than long (ratio: 63:37), anterior lobe broadly subrounded, with single marginal setae (fig. 4A). Coxa 6 smaller than coxa 5, bilobed, broader than long (ratio: 53:33) (fig. 4C). Coxa 7 short, entire, broader than long (ratio: 44:22) (fig. 4E).

Gnathopods 1-2 moderately large, with propodus not distinctly larger than corresponding coxae (fig. 2A, D). Gnathopod 1: article 2 along anterior margin with row of long single setae, along posterior margin with several bunches of long setae often extending diameter of article itself. Article 3 at posterior margin with one distal bunch of setae (fig. 2A); article 5 nearly as long as propodus, along anterior margin with distal bunch of setae, along posterior margin with numerous setae. Propodus trapezoid (Fig. 2B), slightly longer than broad (ratio: 83:74), along posterior margin with 12 transverse rows of setae; palm inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2-3 L-spines and facial 5- 7 M-setae (fig. 2C), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with 11 setae [1-1-1-1-1-3-2-1) or: 1-1-2-3-2-1-1)]; along inner margin are present several short single setae (fig. 2B).

Gnathopod 2: article 2 along anterior margin with row of long single setae, along posterior margin with bunches of long setae (setae are shorter towards distal part of article); article 3 with bunch of longer setae at distoposterior margin (fig. 2D); article 5 almost as long as propodus, along anterior margin with distal bunch of setae, along posterior margin with numerous setae. Propodus trapezoid, rather larger than that of gnathopod 1, hardly longer than broad (ratio: 90:87), along posterior margin with 15 transverse rows of setae; palm inclined nearly half of propodus-length, convex, defined on outer face by corner S-spine accompanied laterally by 2-3 serrate L- spines sitting laterally very close to S-spine, and by 5-7 facial M-setae, on inner face by one subcorner R-spine (fig. 2F). Dactylus reaching posterior margin of propodus, along outer margin with row of 9 single setae, or row of 10 setae (1-1-1-2-3-2); along inner margin appear several short single setae (fig. 2E).

Pereopods 3-4 moderately strong. Pereopod 3: article 2 in proximal part with long anterior and posterior setae, in distal part setae are short and less numerous; articles 4-6 of different length (ratio: 55:33:43), article 4 along both margins with single or groups of short setae; article 5 along posterior margin with single short spines; article 6 along posterior margin with 5 single slender spines (fig. 3E). Dactylus short and strong, much shorter than article 6 (ratio: 16:43), along inner margin with one strong spine near basis of the nail, along outer margin with one median plumose seta; nail nearly as long as pedestal (fig. 3F).

Pereopod 4 similar to pereopod 3, but hardly shorter; pilosity of article 2 like that of pereopod 3; articles 4-6 of different length (ratio: 51:32:41); article 4 along posterior margin with 4 setae and one spine, along anterior margin with 2

median and 3 distal setae; article 5 along posterior margin with 3 single or pair of short spines, along anterior margin with one median and 2-3 distal setae; article 6 along posterior margin with 4 single or pairs of short spines (fig. 3G). Dactylus short and strong, much shorter than article 6 (ratio: 17:41), along outer margin with one median plumose seta, along inner margin with one strong spine near basis of the nail (fig. 3H); nail is hardly shorter than pedestal.(ratio 27:30).

Pereopods 5-7 moderately stout, pereopod 5 distinctly shorter than pereopods 6 and 7. Pereopod 5: article 2 dilated, longer than broad (ratio: 69:43), anterior convex margin provided with row of nearly 6 single or pairs of spine-like setae, along posterior almost straight margin with nearly 18 short setae, ventroposterior part dilated but not lobed (fig. 4A). Articles 4-6 of different length (ratio: 46:47:50), all articles along both margins with single or pairs of short spines; only article 4 along anterior margin with 3 median groups of short setae. Article 2 slightly longer than article 6 (ratio: 69:50). Dactylus much shorter than article 6 (ratio: 15:50), strong, at inner margin with one strong spine near basis of the nail, along outer margin with one median plumose seta (fig. 4B);-nail as long as pedestal.

Pereopod 6: article 2 much longer than broad (ratio: 81:49), anterior convex margin with row of 7 single slender spines; posterior margin slightly concave in the middle, along margin with nearly 16 short setae; ventroposterior dilatation without lobe (fig. 4C). Articles 4-6 of different length (ratio: 59:69:81), along both margins with single or bunches of short spines. Article 2 nearly as long as article 6. Dactylus much shorter than article 6 (ratio: 21:81), along inner margin with strong spine near basis of the nail, along outer margin with one median plumose seta (fig. 4D); nail shorter than pedestal (ratio: 28:50).

Pereopod 7 poorly shorter than pereopod 6; article 2 much longer than broad (ratio: 85:47), along anterior convex margin with row of 6 slender spines, along posterior poorly convex margin with 16 short setae, ventroposterior part of article 2 narrowed (fig. 4E). Articles 4-6 of different length (ratio: 50:63:88), along both margins with bunches of short spines (spines are shorter than diameter of articles themselves). Article 6 poorly longer than article 2 (ratio: 88:85). Dactylus much shorter than article 6 (ratio: 24:88), along inner margin with strong spine near basis of the nail, on outer margin with median plumose seta (fig. 4F); nail shorter than pedestal (ratio: 26:47).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 along anterior margin with 3 single setae (fig. 4G); peduncle of pleopod 2 naked (fig. 4H); peduncle of pleopod 3 along posterior margin with 2 distal and 2 median setae (fig. 4 I).

Coxal gills ovoid, relatively short, not reaching ventral margin of corresponding article 2 (figs. 2D, 3E, G; 4A, C).

Uropods 1-2 rather stout. Uropod 1: peduncle with dorsointernal row of setae (except distal spine) and dorsoexternal row of spines (fig. 3A); rami shorter than peduncle: inner ramus with 2 single lateral spines at inner margin and 2 lateral spines at outer margin (both accompanied by bunch of 3-4 simple setae;

outer ramus only slightly shorter than inner ramus, with 3 lateral spines at inner margin and 2 single spines at outer margin accompanied by 2-4 simple setae each; at tip of rami appear 5 distal short spines (fig. 3A).

Uropod 2: peduncle with row of dorsal spines; inner ramus only poorly longer than outer one, with 3 spines along outer margin and one spine at inner margin, at top with 5 distal short spines; outer ramus with 3 lateral and one median spine; at top are attached 5 distal short spines (fig. 3A).

Uropod 3 long; peduncle longer than broad, with 3-4 distal short spines; inner ramus much shorter than peduncle, bearing 2 distal spines and one plumose seta; outer ramus long, 2-articulated: first article along outer margin with 3 median spines and distal bunch of short spines, along inner (mesial) margin with 5 groups of single or pair of spines accompanied by single plumose setae not exceeding diameter of article itself; second article poorly shorter than first one (ratio: 125:135), along both margins and tip with single simple setae (fig. 5B).

Telson poorly broader than long, incised nearly 2/3 of telson-length; each lobe with 4 distal long spines, one outer marginal spine and 4 median facial spines; left lobe with one mesial marginal spine (fig. 1H).

FEMALE 13.1 mm with 10 eggs in marsupium: Metasomal segments 1-3 with 8-10 dorsoposterior marginal setae (fig. 6F); urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 on each dorsolateral side with 2 spines and 2 setae; urosomal segment 3 naked. Urosomal segment 1 at ventroposterior corner with one weak spine near basis of uropod 1-peduncle (fig. 7E).

Epimeral plates 1-2 obtusely angular, epimeral plate 3 distinctly angular. Epimeral plate 1 with ventral margin slightly concave in the middle and convex posterior margin bearing nearly 10 setae, corner spine-like seta is weak. Epimeral plate 2 with convex ventral margin bearing one submarginal spine, posterior margin poorly convex, bearing nearly 10 setae, including corner weak spine-like seta. Epimeral plate 3 with convex ventral margin bearing 3 subventral spines, posterior margin nearly straight, inclined, bearing nearly 11 setae, including weak corner spine-like seta (fig. 6F).

Head and antennae mainly like these in males. Antenna 1 reaching nearly half of body, main flagellum with 28 articles. Antenna 2 rather more setiferous, peduncular articles 4-5 with longer setae, flagellum with 10 articles (fig. 5C).

Mouthparts mainly like these in male. Mandibles: right mandible incisor and lacinia mobilis like these in male, incisor with 4 teeth and serrate lacinia mobilis accompanied by 8 rakers (fig. 6A). Mandibular palpus article 2 with 20 setae; article 3 subfalciform, slightly longer than article 2, bearing 33 D-setae, 7 E-setae, 2 groups of A-setae (8-1) and 4 groups of B-setae (7-6-5-1).

Maxilla 1 inner and outer plate like these in male, palpus article 2 with 11 setae.

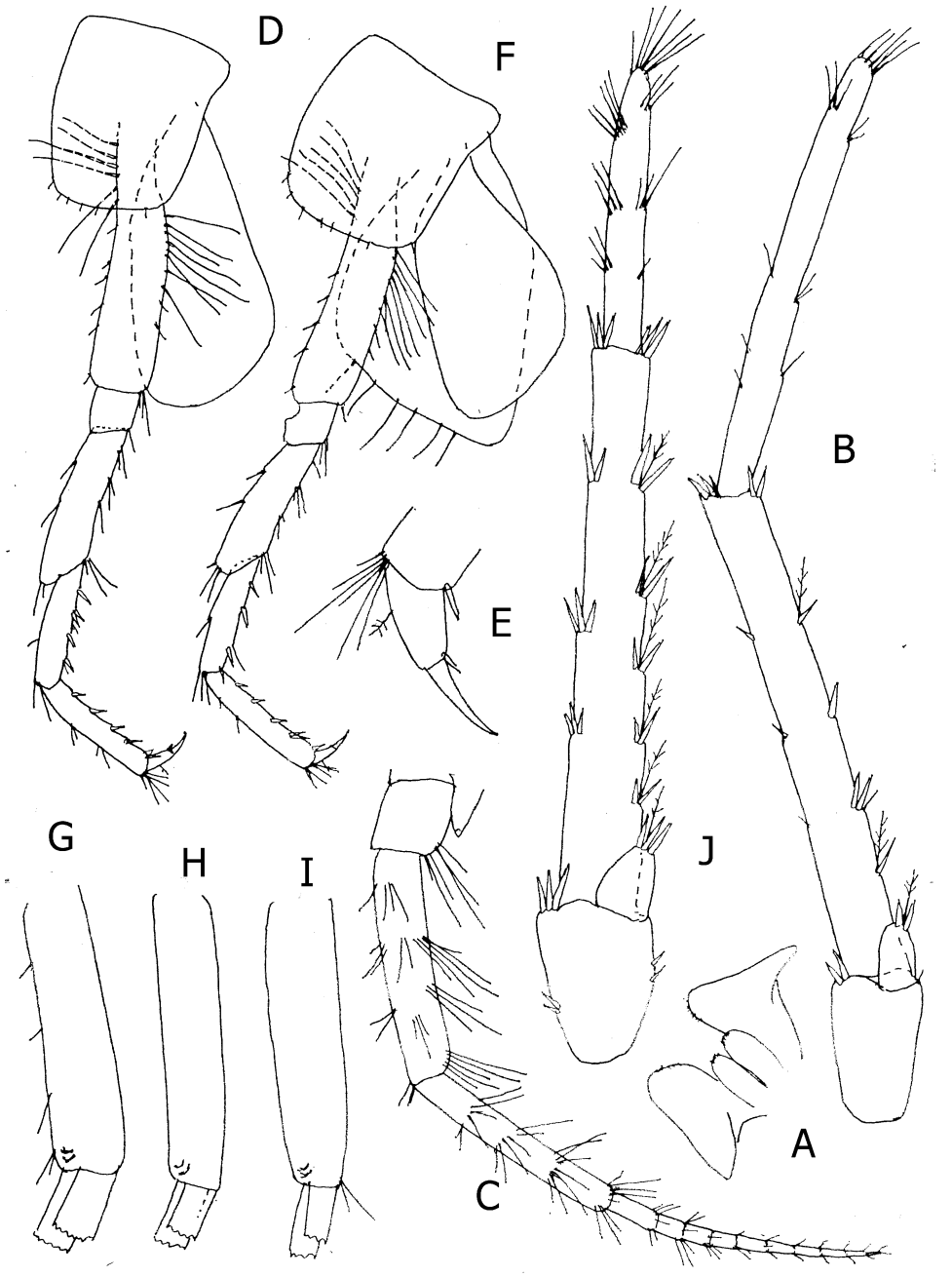

Fig. 5. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Male 17.1 mm: A= labium; B= uropod 3.
Female 15.1 mm: C= antenna 2; D= pereopod 3; E-F= pereopod 4; G= pleopod 3 peduncle; H= pleopod 2 peduncle; I= pleopod 3 peduncle.

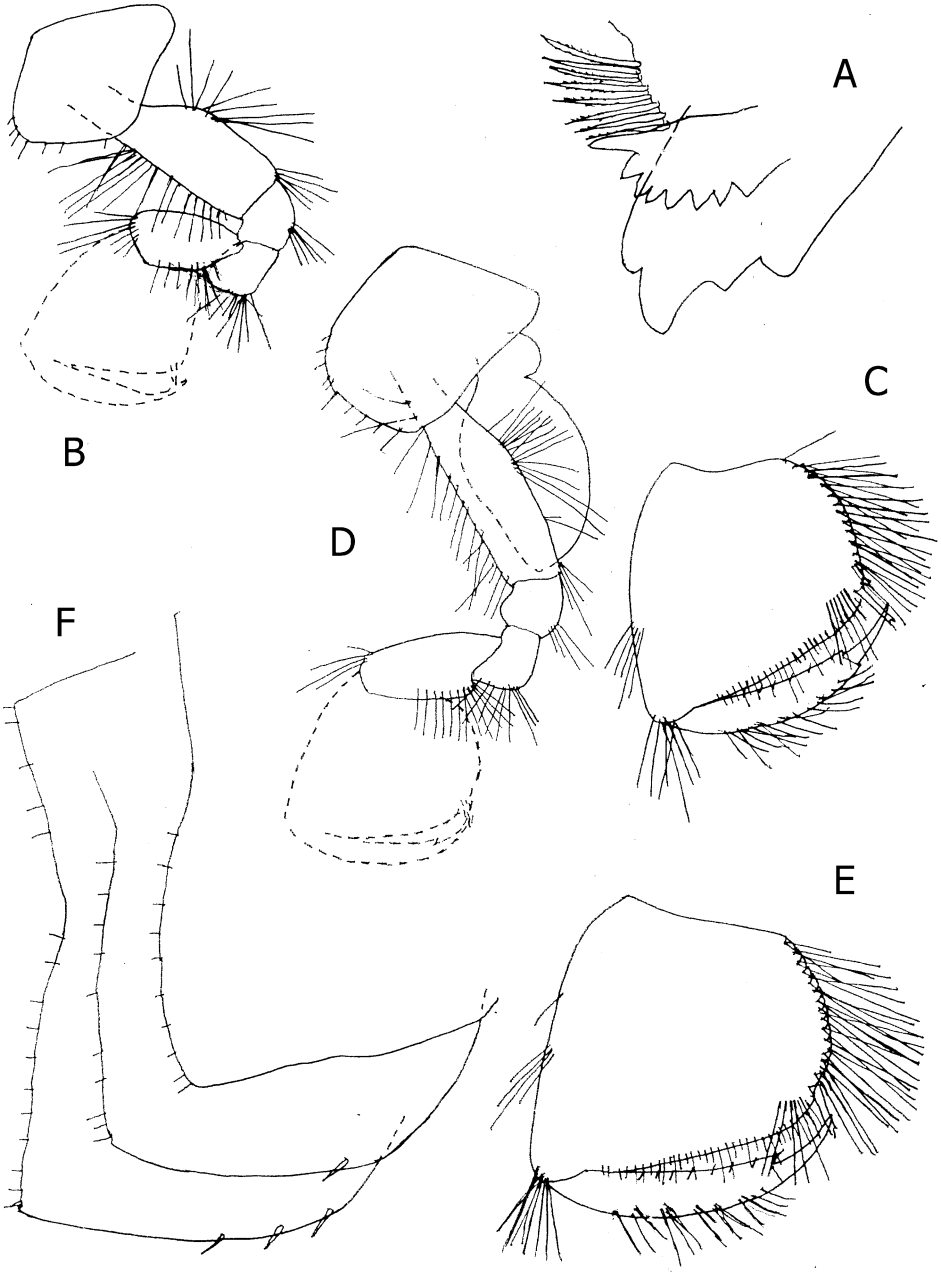

Fig. 6. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Female 15.1 mm: A= right mandible, incisor and lacinia mobilis with rakers; B-C= gnathopod 1; D-E= gnathopod 2; F= epimeral plates 1-3.

Maxilliped: inner plate of left maxilliped with 5 distal spines, that of right maxilliped with 4 distal spines; palpus article 4 with 2-3 setae at inner margin near basis of the nail.

Coxa 1 broader than long (ratio: 40:32) with subrounded ventroanterior part and 7 ventral marginal setae (fig. 6B); coxa 2 longer than broad (ratio: 46:40), ventral margin convex, provided with 11 setae (fig. 6D). Coxa 3 longer than broad (ratio: 52:43), at ventral margin with 7-8 setae (fig. 5D). Coxa 4 poorly longer than broad (ratio: 52:45), with nearly 8 marginal setae, ventroposterior dilatation absent (fig. 5F).

Coxae 5-7 short. Coxa 5 bilobed, much broader than long (ratio: 52:38), anterior lobe subrounded and not produced (fig. 7A). Coxa 6 smaller than 5, bilobed, broader than long (ratio: 42:30) (fig. 7B). Coxa 7 entire, much broader than long (ratio: 40:20) (fig. 7C).

Gnathopods 1-2 of the moderate size, with propodus nearly as large as corresponding coxa (fig. 6B, D). Gnathopod 1 moderately smaller than gnathopod 2; article 2 along anterior margin with row of long single setae, along posterior margin with proximal and distal bunch of setae and several setae in distal part of article; article 3 at posterior margin with distal bunch of setae; article 5 shorter than propodus (ratio: 29:40), along anterior margin with distal bunch of setae (fig. 6B), along posterior margin with numerous setae. Propodus trapezoid, slightly longer than broad (ratio: 67:60), along posterior margin with 14 transverse rows of setae (fig. 6C); palm slightly convex, inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 3-4 serrate L-spines and 6-7 facial M-setae (fig. 6C), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with nearly 13-14 single or paired setae (1-2-1-2-1-1-2-1-1-1), along inner margin with several short single setae.

Gnathopod 2: article 2 along anterior margin with row of numerous long setae, along posterior margin with proximal bunch of setae and several setae in distal part. Article 3 at posterior margin with distal bunch of setae (fig. 7D); article 5 slightly shorter than propodus (ratio: 37:40), along anterior margin with distal bunch of setae, along posterior margin with numerous setae. Propodus trapezoid, only slightly longer than broad (ratio: 75:70), along posterior margin with 14 transverse rows of setae; palm slightly convex, inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 2 L-spines and 6 facial M-setae, on inner face by one subcorner R-spine (fig. 7E). Dactylus reaching posterior margin of propodus, along outer margin with nearly 13-14 single or paired setae (1-2-3-2-2-2-1-1), along inner margin with row of short setae.

Pereopod 3 moderately strong; article 2 in proximal part with numerous long setae at both margins (fig. 5D) and short setae in distal part. Articles 4-6 of different length (ratio: 43:30:37); article 4 along both margins with single or pairs setae not exceeding diameter of article itself; article 5 along posterior margin with 4 single spines mixed with single short seta, along anterior margin with single

setae; article 6 along posterior margin with 5 single spines sometimes with one short seta, along anterior margin with several short setae. Dactylus much shorter than article 6 (ratio: 21:37), along inner margin with one spine near basis of the nail, along outer margin with one median plumose seta; nail shorter than pedestal (ratio: 26:32) (fig. 5E).

Pereopod 4: article 2 in proximal part along both margins with row of long setae, and short setae in distal part; articles 4-6 of different length (ratio: 40:30:36); article 4 along both margins with short setae; article 5 along posterior margin with 3 groups of 1-2 spines and single setae; article 6 along posterior margin with row of 5 single spines mixed sometimes with single short seta (fig. 4F); dactylus much shorter than article 6 (ratio:16:36), along inner margin with one spine near basis of the nail, along outer margin with one median plumose seta; nail like that in pereopod 3.

Pereopod 5 remarkably shorter than pereopods 6 and 7 (fig. 7A, B, C), article 2 much longer than broad (ratio: 58:32), with almost parallel lateral margins, along anterior slightly convex margin appear a row of 6 single spine-like setae and distal bunch of setae, posterior almost straight margin provided with nearly 15 short setae; ventroanterior part not produced, ventroposterior dilatation isn`t lobed (fig. 7A). Articles 4-6 of different length (ratio: 44:49:51), all these articles along both margins with short spines, sometimes mixed with single short seta. Article 2 is longer than article 6 (ratio: 58:51). Dactylus much shorter than article 6 (ratio: 16:51), along inner margin with one spine near basis of the nail, along outer margin with one median plumose seta, nail shorter than pedestal.

Pereopod 6: article 2 much longer than broad (ratio: 72:39), weakly tapering ventrally, along anterior weakly convex margin appear a row of 8 short spine-like setae, along posterior almost straight margin are attached 13 short setae; ventroposterior dilatation not forming lobe (fig. 7B). Articles 4-6 of different length (ratio: 50:67:76), along both margins of these articles appear bunches of single or paired spines accompanied often with single short setae (fig. 7B). Article 2 is poorly shorter than article 6 (ratio: 72:76). Dactylus much shorter than article 6 (ratio: 27:76), along inner margin with one spine near basis of the nail, along outer margin with one median plumose seta, nail shorter than pedestal.

Pereopod 7 scarcely shorter than pereopod 6; article 2 much longer than broad (ratio: 75:41), along anterior slightly convex margin appear 7 single spine-like setae, along posterior slightly convex margin appear nearly 13 short setae, ventroposterior corner without lobe. Articles 4-6 of different length (ratio: 45:55:75), along both margins with short spines mixed sometimes with single short seta (fig. 7C). Article 2 nearly as long as article 6. Dactylus much shorter than article 6 (ratio: 27:75), along inner margin with spine near basis of the nail, along outer margin with one median plumose seta; nail shorter than pedestal (ratio: 27:50) (fig. 7D).

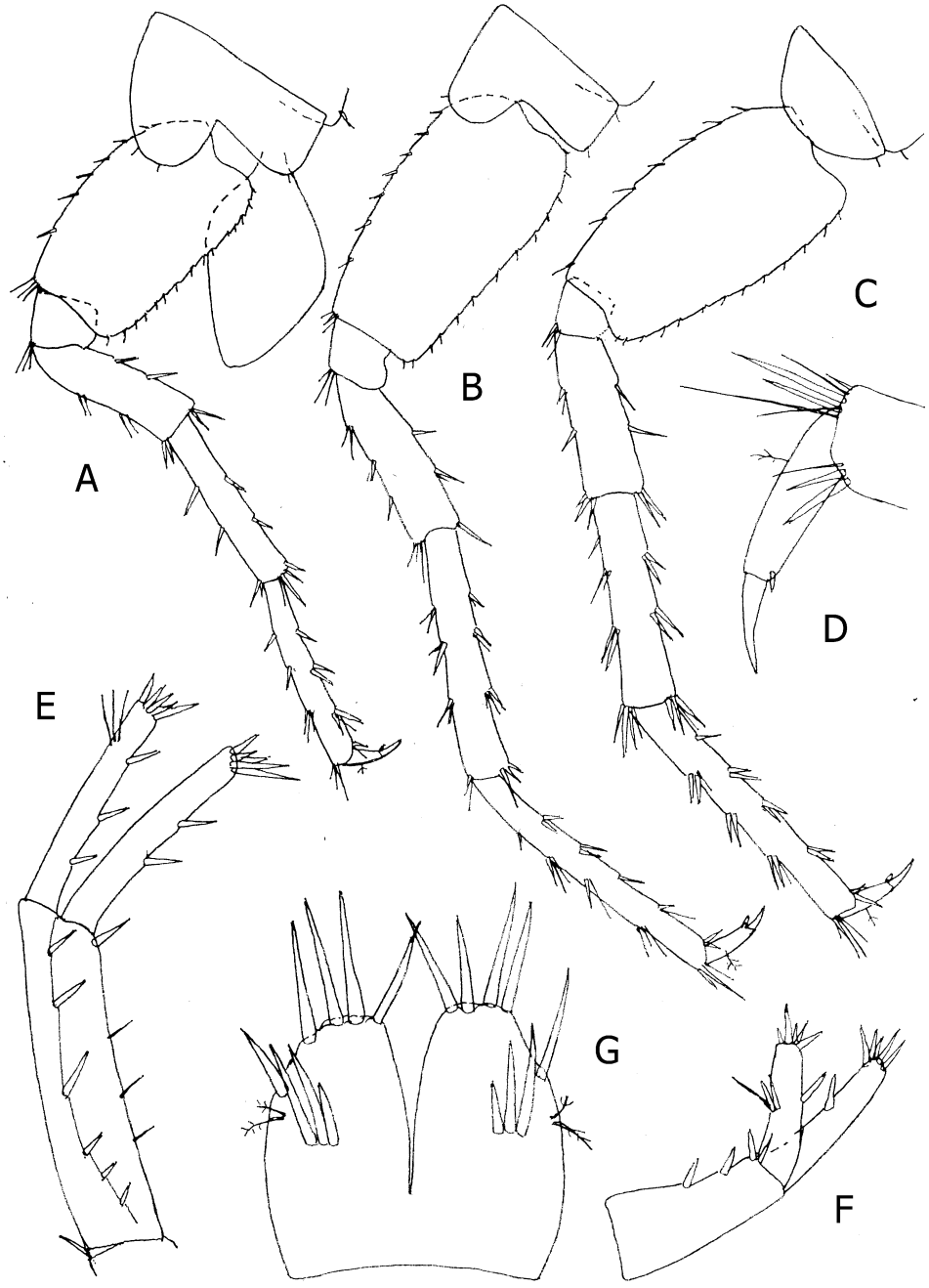

Fig. 7. *Niphargus julius* Stoch, 1997, Grotta Nuova di Villanova Cave, Female 15.1 mm: A= pereopod 5; B= pereopod 6; C-D= pereopod 7; E= uropod 1; F= uropod 2; G= telson.

Pleopods 1-3 with 2 retinacula. Peduncle of pleopod 1 with 2 distal simple setae (fig. 5 G); peduncle of pleopod 2 naked (fig. 5H); peduncle of pleopod 3 with 4-5 short simple setae along posterior margin (fig. 5 I).

Uropods 1-2 stout. Uropod 1 rami shorter than peduncle. Peduncle with dorsointernal row of setae (except distal spine), and dorsoexternal row of spines (fig. 7E). Inner ramus only slightly longer than outer one, with 2-3 lateral and 5 distal short spines. Outer ramus with 3 lateral spines and one subdistal bunch of simple setae, at top appear 5 distal short spines.

Uropod 2: peduncle with 4 dorsal spines; inner ramus very poorly longer than outer one, with one lateral and 5 distal spines; outer ramus with 2 lateral spines and one simple seta at outer margin, and 2 single spines at mesial margin (fig. 7F); at top are attached 5 unequal spines.

Uropod 3 long, but shorter than that of male. Peduncle slightly longer than broad, with 2 lateral spines and setae and 3 distal spines; inner ramus scale-like, much shorter than peduncle (fig. 5J) and provided with 3 distal spines. Outer ramus 2-articulated: first article long, along outer margin with 4 bunches of short spines, along inner (mesial) margin with 6 bunches of spines mixed with single plumose setae longer than spines; distal article long but shorter than first one (ratio: 77:150), along both margin and tip with several bunches of simple setae (fig. 5J).

Telson only slightly broader than long, incised nearly 2/3 of telson-length; each lobe with 4 distal and one outer marginal longer spine; a bunch of 3 median facial spines attached on each lobe; a pair of short plumose setae is attached in the middle of outer margin of each lobe (fig. 7G).

Coxal gills broad, but not exceeding ventral margin of corresponding article 2 (gnathopod 2, pereopods 3 and 4) (figs. 5D, F; 6D), or shorter (pereopods 5 and 6) (fig. 5A).

Oostegites very broad, with marginal setae (fig. 5F).

VARIABILITY.

Females rather similar to males, but with slightly longer anterior coxae. Metasomal segmens 1-3 with 8-10 dorsoposterior marginal setae.

Dactylus of all pereopods always with strong spine at inner margin near basis of the nail; Dactylus of gnathopods 1-2 along outer margin mainly with row of single setae, but often appear some groups of 2 setae, or rarely 3 setae. Antenna 2 in female slightly more setose than that in male. The males and females have similar shape of epimeral plates.

Stanko Karaman figured 3 different telsons showing the variability in number of spines and proportion of telsons: telson as long as broad, telson poorly longer than broad and telson poorly broader than long (S. Karaman, 1954, figs. 9-11).

The male 15.5 mm from Punt de Pratis: propodus of gnathopods 1 and 2 with corner S-spine accompanied laterally by 3 L-spines; and 6-7 M-setae; setae along outer margin of dactylus in gnathopods 1-2 like these in holotype of

Villanova. Maxilliped: inner plate with 3 and 4 distal spines, palpus article 4 at inner margin with 2 setae near basis of the nail.

REMARKS AND AFFINITIES.

Stanko Karaman (1954) underlines main differences of this species regarding *N. stygius* and some other similar populations of *Niphargus stygius*-Complex from N. Italy and Slovenia.

Based on present knowledge of this complex we can mentioned some of them regarding *N. julius*.

N. stygius stygius (Schiödte, 1847) [loc. typ.: Postojnska jama Cave, Slovenia] differs distinctly from *N. julius* by absence of facial spines on telson, by shorter distal article of uropod 3 outer ramus in female, maxilla 1, etc.

Niphargus valvasori S. Karaman 1952 [loc. typ.: Križna Jama Cave, Slovenia] differs from *N. julius* by different maxilla 1, by absence of facial spines on telson, etc.

N. costozzae Schellenberg 1935 [loc. typ.: "Covolo della guerra" Cave, Lumignano, Monti Berici Mt., N. Italy, is also rather similar to *N. julius*, but differs by less spiniferous telson, elevated number of setae on maxilla 1 inner plate, presence of spine on urosomal segment 1, etc.

From northern Italy have been described several other similar species [*N. dolenianensis* Lorenzi 1898 [loc. typ. Dolegnano, between Udine and Gorizia], *N. lessiniensis Stoch* 1998 (loc. typ.: Lessinian Mts, Verona), *N. stebbingi* Cecchini 1928 [loc. typ.: Poggio degli Balzi, Toscana], *N. tridentinus* Stoch 1998 (loc. typ.: Grotta delIa Bigonda" Cave, Grigno, Trento), *N. montellianus* Stoch 1998 [loc. typ.: Massif of Montello, Venetia] etc., but these species differs from *N. julius* by various characters, although some of them are very scarcely described and needs verification and detailed description. As the entire *Niphargus stygius*-Complex from northern Italy is still in the process of study, we have given up here further comparison with other taxa of this group. Probably the new molecular genetics study of all these taxa will put more light on the problem of delimitation of all these taxa.

LOCUS TYPICUS: Grotta Nuova di Villanova Cave, Fr 323, N. Italy.

LOCALITIES CITED:

S. Karaman (1954) cited this species for Cave Villanuova and Puint de Pratis. Stoch (2008) mentioned this species in numerous localities as author (several papers) or as coauthor with S. Ruffo (2005), Dorigo (2007) and Colla (2002): Buse da l'Ors-Cave (Fr 64), Grotta del Cret dal Landri-Cave (Fr 58), Grotta di Monteprato-Cave (Fr 59), Grotta di Taipana-Cave (Fr 61), Grotta Furmie-Cave (Fr 187), cave of quarry in Papipano (Fr 296), Grotta Pod Lanisce-Cave (Fr 573), Grotta di Canebola-Cave (Fr 1080), Grotta Doviza-Cave (Fr 70), Grotta Egidio Feruglio-Cave (Fr 2175), Grotta Nuova di Villanova-Cave (Fr 323), Grotta Pre Oreak-Cave (Fr 65), spring "sul sentiero 727" (Fr 2786); springs

of Torlano water system; springs on left bank of torrent Vedronza in the valley of Casera Morandin.

DISTRIBUTION: *Niphargus julius* is endemic species of NE part of Italy (Prealpi Giulie, Venezia Giulia).

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In memoriam

Prof. dr Slavko Mijović
(1954-2020)

Dana 15. oktobra 2020. godine, iznenada, u 66 godini života, preminuo je naš dragi prijatelj i kolega prof. dr Slavko Mijović, dugogodišnji naučni radnik i profesor na Biotehničkom fakultetu Univerziteta Crne Gore.

Slavko Mijović je rođen 24.10.1954. godine u Golubovcima, u poljoprivrednoj porodici. Osnovnu školu završio je u rodnim Golubovcima, a Srednju građevinsku školu u Podgorici. Poljoprivredni fakultet, Odsjek za voćarstvo i vinogradarstvo, završio je u Novom Sadu 1980., kao i magistarske studije 1986. godine.

Doktorsku disertaciju iz oblasti vinogradarstva odbranio je na Agronomskom fakultetu u Čačku 1993. godine.

Prvi radni angažman u struci započeo je odmah po završetku fakulteta, 1980. godine, kada je zasnovao radni odnos u R.O. "Plantaže" kao pripravnik u vinogradarskoj proizvodnji. Tu je proveo tri godine, nakon čega prelazi u Agroekonomski institut, gdje je učestvovao u realizaciji brojnih projekata iz oblasti vinogradarstva.

Na Poljoprivrednom institutu u Podgorici radni odnos je zasnovao 1987. godine gdje je radio kao stručni saradnik. Istovremeno je nastavio svoje naučno-stručno usavršavanje, kroz specijalizacije i studijske boravke u zemlji i inostranstvu (SSSR, Bugarska i dr.). Bio je rukovodilac većeg broja projekata iz oblasti vinogradarstva i redovni učesnik brojnih naučnih skupova u zemlji i inostranstvu. Na Univerzitetu Crne Gore prošao je sve izborne procedure, od višeg istraživača do naučnog savjetnika.

U više navrata obavljao je funkciju šefa Zavoda za voćarstvo i vinogradarstvo, i to u periodu od 1994. do 1998. i od 2001. do 2008. godine. U periodu od 1998-2001. godine bio je pomoćnik ministra Poljoprivrede, šumarstva i vodoprivrede, a funkciju pomoćnika direktora Biotehničkog instituta obavljao je u periodu od 2004. do 2007. godine.

Od uspostavljanja studija iz oblasti poljoprivrede (2005. godine) i transformisanja Biotehničkog instituta u Biotehnički fakultet bio je angažovan

kao predavač na predmetima iz oblasti vinogradarstva. Tokom tog perioda bio je mentor na više od 40 specijalističkih i 7 magistarskih radova.

Dr Slavko Mijović je kao autor ili koautor objavio dvije knjige i dva praktikuma i više od 150 naučnih radova. Učestvovao je u stvaranju dvadeset stonih sorti vinove loze, koje predstavljaju ogromno bogatstvo ne samo za Biotehnički fakultet, nego i za čitavu Crnu Goru.

Tokom svoje profesionalne karijere svo svoje znanje i organizacione sposobnosti posvećivao je Oglednom imanju ‘’Lješkopolje’’ gdje je aktivno učestvovao u podizanju zasada i uvođenju novih tehnologija u proizvodnji grožđa i vina. Pored rada na Oglednom imanju, aktivno je učestvovao i u podizanju brojnih zasada širom vinogradarskog regiona Crne Gore.

Za nesebično angažovanje i uspješan rad dobio je više društvenih priznanja. Poslednje u nizu bilo je nagrada „8. februar” koju mu je dodijelila Gradska opština Golubovci 2019. godine za dostignuća ostvarena u naučno-istraživačkom radu i doprinos unapređenju razvoja poljoprivrede u Crnoj Gori.

Dr Slavko Mijović je bio izuzetno skroman čovjek koji je volio da pomaže drugima. Njegovim odlaskom izgubili smo vrijednog i poštenog kolegu i prijatelja. Inspirisao nas je svojim znanjem, mudrošću i čovječnošću i zbog toga će vječno ostati u našim srcima.

Iza sebe je ostavio suprugu, sina, unučad, brojnu rodbinu i prijatelje koji će se sa sjetom sjećati njegovog dragog lika.

Dragi Slavko, u ime kolega sa Biotehničkog fakulteta, hvala ti na razumijevanju, na svakoj toploj riječi, savjetu i svemu ljudskom po čemo ćemo te pamtiti.

Neka Ti je vječna slava i hvala!

Dr Tatjana Popović

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