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CONTENT

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PRIMING WITH NO DONOR SODIUM NITROPRUSSIDE TO ACTIVATE GERMINATION AND REDUCE OXIDATIVE DAMAGE IN AGED WHEAT AND TRITICALE SEEDS

SUMMARY

Despite the existence of numerous experimental data on the stimulation of seed germination of various plant species under unfavourable conditions by nitric oxide donors, there are very few data on the effect of exogenous NO on the germination of cereal seeds that have undergone accelerated ageing. In particular, they are lacking for aged seeds of one of the most widespread bread cereals, *Triticum aestivum* L. Any physiological effect of NO on the wheat-rye hybrid triticale (×*Triticosecale*) has not been studied. The aim of this work was to study the effect of priming aged wheat seeds of cultivar Scorpion and triticale seeds of cultivar Raritet with the nitric oxide donor sodium nitroprusside (SNP) on their germination and seedling growth parameters. A three-hour treatment of seeds with SNP (optimum concentration 100 µM) resulted in increased germination energy, germination, and accumulation of shoot and root biomass in both species. The effects of SNP were almost completely eliminated by the action of the NO scavenger methylene blue. Moreover, there was no effect of SNP on seed germination and seedling growth subsequent to its preliminary decomposition under light conditions. This suggests that the physiological activity of SNP may be linked to the release of nitrogen oxide. Treatment of seeds with SNP resulted in a reduction in the generation of superoxide anion radicals and hydrogen peroxide in the shoots of seedlings of both species, as well as a reduction in the content of malonic dialdehyde, a product of lipid peroxidation. Concomitantly, alterations in the activity of pivotal antioxidant enzymes (superoxide dismutase and catalase) were observed in seedlings derived from seeds treated with SNP.

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Furthermore, the application of SNP to seeds resulted in an increase in the total phenolic compound content in wheat seedlings and the amount of anthocyanins in triticale seedlings. It was concluded that there is a correlation between the ability of exogenous NO to mitigate oxidative damage and enhance germination of aged cereal seeds.

Keywords: *Triticum aestivum,* × *Triticosecale*, seed aging, germination, nitric oxide, oxidative stress, antioxidant system

INTRODUCTION

Nitric oxide (NO) is an important gasotransmitter molecule at the centre of the signalling network of plant and animal cells (Sougrakpam *et al*., 2023; Khator *et al*., 2024). Nitric oxide is known to be involved in the regulation of multiple functions of the plant organism: seed germination, flowering, pollen formation, fruit ripening and senescence, as well as legume-rhizobium symbiosis and responses to biotic and abiotic stressors (Turkan, 2017; Venkatesan *et al.*, 2020; Singhal *et al.*, 2021). The molecular basis for these diverse physiological effects of it is the process of post-translational modification of many proteins, as well as direct and indirect interactions with other key players in the signalling network (primarily reactive oxygen species (ROS) and calcium ions) and many plant hormones (Sami *et al*., 2018; Khan *et al.*, 2023; Sougrakpam *et al.*, 2023).

One of the important biological effects of nitric oxide, which is not only of fundamental but also of applied importance, is its ability to stimulate the germination of seeds of various plant species. The activation of seed germination of some plant species by inorganic and organic nitrates was discovered at the end of the last century (Grubisic and Konjevic, 1990; Grubisic *et al.*, 1992). Later, the effect of NO donors on dormancy interruption and seed germination was studied in detail using the model species *Arabidopsis thaliana* L. (Bethke *et al.*, 2004). To date, this phenomenon has been documented in experiments on the effect of nitric oxide donors on seed germination of plants of different taxonomic affiliation, in particular lettuce (*Lactuca sativa* L.), yellow lupin (*Lupinus luteus* L.), tomato (*Solanum lycopersicum* L.), barley (*Hordeum vulgare* L.), millet (*Panicum virgatum* L.) and other cultivated plant species (see reviews: Bethke *et al.*, 2007; Krasuska *et al.*, 2015). The applied aspect of these studies is mainly related to the search for ways to increase seed germination energy in species whose seeds do not germinate uniformly due to biological peculiarities (Sarath and Mitchell, 2008). The effect of NO donors on seed germination under stress conditions has also been widely studied (Duan *et al*., 2007; Sepehri and Rouhi, 2016). One of the main mechanisms of the beneficial effect of nitric oxide on seed germination is considered to be its modification of the hormonal balance in germinating seeds, associated with the inhibition of abscisic acid synthesis enzymes (the hormone responsible for seed dormancy) under the action of NO and the activation of enzymes providing synthesis of gibberellic acid and ethylene, factors promoting seed germination (Šírová *et al*., 2011; Arc *et al.*, 2013; Kolbert *et al.*, 2019b, Kolupaev *et al.*, 2024b).

To increase the speed and uniformity of seed germination, especially under unfavourable conditions, priming technologies have been used quite actively in the last decade (Janmohammadi, 2012; Paparella *et al.*, 2015; Sako *et al*., 2020). The term 'seed priming' usually defines a method of controlled wetting and drying of grains to enhance pre-germination metabolic processes in them (Waqas *et al*., 2019). The simplest priming technique, hydropriming, is more commonly used in combination with seed exposure to solutions of various physiologically active compounds, mainly plant hormones and signalling mediators (Ibrahim, 2019; Sen *et al.*, 2021), including NO (Duan *et al*., 2007; Kaur and Kaur, 2018; Kumar *et al.*, 2021).

Long-term storage is known to reduce seed germination and viability due to ageing. Preservation of seed germination for a certain period depends on both species characteristics and storage conditions. It is known that storing seeds at higher humidity and temperature accelerates ageing, which eventually leads to loss of viability (Probert *et al.*, 2007). One of the causes of accelerated seed ageing under inappropriate storage is the spontaneous formation of ROS in mitochondria and other organelles (Ratajczak *et al.*, 2019) and, as a consequence, lipid peroxidation (LPO), which leads to changes in the composition, structure and function of biomembranes (Kurek *et al*., 2019; Lin *et al.*, 2022). The development of oxidative stress also causes protein carbonylation characteristic of ageing seeds (Rajjou *et al*., 2008) and, in some cases, DNA damage (Kurek *et al.*, 2019; Afzal, 2023).

It has also been shown that the onset of seed germination is often accompanied by an increase in the generation of ROS, which are involved in the generation of redox signals necessary for seedling growth (Kranner *et al*., 2010). However, in ageing seeds there is usually an imbalance between ROS generation and their neutralisation by the antioxidant system (Zhang *et al.*, 2021), which may be responsible for oxidative damage to the structures of the developing seedling. In this regard, it has been suggested that priming aged seeds with antioxidants may improve their germination. In particular, classical antioxidants such as reduced glutathione and ascorbic acid have been shown to improve germination of aged oat seeds (Xia *et al*., 2020). Priming with melatonin, which has direct and indirect antioxidant effects, improved germination of aged rye and triticale seeds (Kolupaev *et al.*, 2024a).

The ability of nitric oxide to alter redox homeostasis is well established (Arnao and Hernández-Ruiz, 2019; Kolbert *et al*., 2019a; Kolupaev *et al*., 2023a; Dey *et al.,* 2024). Although the mechanisms of such effects are not fully understood, nitric oxide can also have pro-oxidant effects, causing so-called nitrosative stress (Corpas and Barroso, 2013). It is known that, depending on the concentration, nitric oxide can cause various post-translational modifications (nitrosation, tyrosine nitration, metallonitrosylation) in the molecules of antioxidant enzymes, leading to modulation of their activity (Arora *et al*., 2016; Singhal *et al*., 2021; Mukherjee and Corpas, 2023). Nitric oxide also affects the activity of enzymes that regulate the pool of reducing agents (Corpas *et al.*, 2013). Finally, nitric oxide can modulate the activity of the key ROS-generating enzyme, NADPH oxidase (Yun *et al.*, 2011). The direct effect of NO on ROS levels and redox homeostasis is combined with its mediated effect (involving other signalling mediators) on the gene expression of antioxidant enzymes, which usually results in enhanced antioxidant activity of plant cells (Correa-Aragunde *et al*., 2015; Kolupaev *et al*., 2023a).

The influence of nitric oxide on seed germination is largely attributed to the modulation of redox homeostasis. Models of an 'oxidative window' and a 'nitrosative door' have been proposed, according to which ROS and reactive nitrogen species (RNS) in a certain concentration range can act as signalling molecules necessary for the induction of metabolic changes responsible for the transition of seeds from dormancy to germination (Krasuska *et al*., 2015). It is thought that seeds remain dormant at low levels of ROS and RNS in the cells, whereas an increased concentration of ROS and RNS leads to seed senescence (Krasuska *et al*., 2015).

Nevertheless, there are very few experimental data on the effect of NO donor priming on the germination of aged seeds of cultivated plants and the state of their antioxidant system. In particular, purely phenomenological data have been obtained on the enhancement of germination of millet seeds with reduced germination due to long-term storage by the NO donor sodium nitroprusside (SNP) (Sarath and Mitchell, 2008). It was shown that SNP treatment improved the germination of aged sunflower seeds and reduced the content of the LPO product malonic dialdehyde (MDA), while no significant changes in the activity of antioxidant enzymes were observed (Pereira *et al.,* 2022). It was also shown that treatment of aged oat seeds with SNP increased their germination, which was accompanied by improved functioning of the mitochondrial tricarboxylic acid cycle, increased activity of ascorbate-glutathione cycle enzymes and activation of alternative oxidase (Mao *et al.,* 2018).

However, for seeds of one of the most important crops, bread wheat (*Triticum aestivum* L.), there are only data on SNP-induced enhancement of germination of seeds with normal germination ability against the background of its inhibition by osmotic (Hua *et al*., 2003), salt (Duan *et al.,* 2007) or cold (Bibi *et al.,* 2020) stress. These effects were accompanied by modulation of antioxidant enzyme activity and a reduction in the manifestation of oxidative stress effects. To date, there is a complete lack of information on the effect of NO donors on the germination of triticale seeds, a hybrid species obtained by crossing wheat and rye and characterised by a rather rapid decrease in seed germination when stored under suboptimal conditions (Kolupaev *et al.,* 2024b).

In this context, the aim of the work was to study the effect of the nitric oxide donor SNP on the germination of wheat and triticale seeds subjected to natural ageing during storage under uncontrolled conditions, and the relationship between the physiological effects of exogenous NO and changes in the state of the antioxidant system of germinating seeds.

MATERIAL AND METHODS

Plant materials and treatments

The experiments were carried out in the Laboratory of Plant Physiology and Biochemistry of the Yuriev Plant Production Institute of NAAS of Ukraine. Seed samples were provided by the National Center for Genetic Resources of Plants of Ukraine. Seeds of winter bread wheat (*Triticum aestivum* L.) of the Scorpion cultivar (Czech Republic, Austria) and winter triticale (× *Triticosecale* Wittmack) of the Raritet cultivar (Ukraine) of the 2020 generation were used for the experiments. The wheat cultivar Scorpion has blue grains with increased content of polyphenolic compounds (Martinek *et al*., 2013). However, it is reported that the seeds of this cultivar, even when properly stored, shrink and shrivel easily, resulting in reduced germination (Martinek *et al*., 2013). Triticale seeds of the Raritet cultivar also lose germination significantly after several years of storage, as shown by previously obtained data (Kolupaev *et al.*, 2024a). Prior to the experiments, wheat and triticale seeds were stored indoors for three years under uncontrolled conditions (in summer the temperature periodically reached 30–32°C, in winter it dropped to -6...-8°C; relative humidity during storage repeatedly changed from 25–30 to 80–85%). As a result, seed germination during 3 years of storage decreased to about 50% for wheat and 40% for triticale.

Seeds from all experimental variants were disinfected with 5% sodium hypochlorite solution for 15 min and washed repeatedly with sterile distilled water. Some seeds were then kept in glasses with distilled water for 3 hours (hydropriming). Hydropriming has previously been shown to increase seed germination slightly (approximately within 10%) (Kolupaev *et al.*, 2024a). Therefore, the hydropriming variant was considered as the control variant.

In the SNP treatment variants, seeds were incubated for 3 h in solutions at concentrations of 20, 100, and 500 μM in diffused light at 24°C. In a separate series of experiments to demonstrate the specific effect of SNP as a nitric oxide donor on seed germination, a so-called "exhausted" SNP solution (concentration 100 μM), containing the degradation products of this compound but having lost NO, was used for comparison. Such an "exhausted" SNP solution was obtained by incubating it in an open dish in the light for 2 days (Kolupaev *et al.*, 2020a). In addition, variants with seed treatment with the NO scavenger methylene blue (Zhang *et al.*, 2006) and a mixture of SNP with methylene blue were included in the experiment. In these experimental variants, the seed priming time was also 3h.

Seeds hydroprimed or treated with SNP or other compounds mentioned above were dried in a dark thermostat at 24°C and 40% humidity for one day. The seeds were then placed in Petri dishes with two layers of sterile filter paper moistened with distilled water and germinated in a dark thermostat at 24°C for 3 days. After 2 days of germination, the relative number of germinated seeds (germination energy index) and the mass of shoots and roots of seeds with normal germination were evaluated. On the 3rd day of germination, the germination index (relative number of germinated seeds) was evaluated. The shoots from normally germinated seeds were used for biochemical analyses.

Superoxide anion radical generation

The generation of superoxide anion radicals (SAR) by the shoots was estimated by nitroblue tetrazolium (NBT) reduction. Ten identical shoots were placed for 1 h in a tube containing 5 ml of 0.1 M K, Na-phosphate buffer (pH 7.6) with 0.05% NBT, 10 μM EDTA, and 0.1% Triton X-100 (Kolupaev *et al.*, 2023b). At the end of the exposure, the shoots were gently removed from the incubation solution. The optical density of the incubation solution was also measured at 530 nm using a UV-1280 spectrophotometer (Shimadzu, Japan). The rate of $O_2^{\bullet-}$ generation was calculated in standard units (A_{530} • 1000/weight of shoot raw material).

Evaluation of hydrogen peroxide content

For the determination of H_2O_2 content, seedling shoots were homogenised in cold with 5% trichloroacetic acid (TCA). Samples were centrifuged at 8000×g for 10 min at 2-4°C in an MPW 350R centrifuge (MPW MedInstruments, Poland). The concentration of H_2O_2 in the supernatant was measured by the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this purpose, 0.5 ml of 2.5 M ammonium thiocyanate, 0.5 ml of 50% TCA, 1.5 ml of supernatant and 0.5 ml of 10 mM ferrous ammonium sulphate were added to tubes. After mixing, the samples were transferred to cuvettes and the absorbance at 480 nm was determined.

Measurement of LPO products content

For the analysis of the amount of LPO products reacting with 2-thiobarbituric acid (mainly malonic dialdehyde, MDA), shoots were homogenised in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% TCA and the homogenate was placed in tubes with foil lids in a boiling bath for 30 min. The samples were then cooled and centrifuged at 10000×g for 15 min. The absorbance of the supernatant was determined at 532 nm (maximum light absorption of MDA) and 600 nm (to correct for non-specific light absorption) (Kolupaev *et al.*, 2020b).

Antioxidant enzyme activity assessment

Seedling samples were homogenised in cold 0.15 M K, Na-phosphate buffer (pH 7.6) with the addition of EDTA (0.1 mM) and dithiothreitol (1 mM). The homogenate was centrifuged at 8000×g for 15 min at a temperature of not more than 4°C to prepare the supernatant, which was then assayed (Kolupaev *et al.*, 2022a). Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined at pH 7.6 by a method based on the ability of the enzyme to compete with nitroblue tetrazolium for superoxide anions generated by the aerobic interaction between NADH and phenazine methosulphate. Catalase (EC 1.11.1.6) was evaluated by the amount of H_2O_2 decomposed per unit time. Guaiacol peroxidase (EC 1.11.1.7) activity was estimated at pH 6.2 using guaiacol as hydrogen donor and H_2O_2 as substrate.

Determination of phenolic and anthocyanin content

For the determination of total phenolic compounds and anthocyanins, seedlings (300 mg) were homogenised in 6 ml of 80% ethanol. The extraction was carried out at room temperature for 20 min. The samples were centrifuged at

8,000 × g for 15 min. To evaluate the content of phenolic compounds, 0.5 ml of the supernatant, 8 ml of distilled water and 0.5 ml of Folin's reagent were added to the reaction tubes and stirred; 3 min later, 1 ml of 10% sodium carbonate was added. After 1 hour, the absorbance of the reaction mixture was measured at 725 nm (Bobo-García *et al.,* 2015). The content of phenolic compounds was expressed as µmol gallic acid/g dry weight.

Before the determination of anthocyanin content, the supernatants were acidified with HCl to a final concentration of 1%. Absorbance was measured at 530 nm (Nogués and Baker, 2000). The results were expressed as A_{530}/g dry weight.

Replication of experiments and statistical processing of data

To determine the impact of seed treatment with SNP and other compounds on seed germination and seedling biomass, each experimental variant was replicated at least three times, with each replicate consisting of 60 seeds. For biochemical analyses, each sample consisted of at least 12 seedlings, with analyses performed in three or more replicates.

The statistical analysis of the results was conducted using the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. The figures and table present the mean values from three biological replicates, along with their standard errors. Different letters indicate values that are significantly different at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Effect of SNP on seed germination and seedling growth parameters

The germination rates of wheat and triticale seeds exposed to temperature and humidity variations during 3 years of storage were 51.4 and 44.3%, respectively, in the control (Figure 1A). SNP priming in the concentration range of 20–500 µM caused an increase in germination energy of seeds of both species. The maximum effect was observed when seeds were primed with 100 µM SNP. A significant increase in seed germination index after SNP treatment was also observed across the range of concentrations used, with a maximum at 100 µM (Figure 1A).

Seed treatment with SNP also resulted in an increase in biomass accumulation of shoots and especially roots of seedlings (Figure 1B). Thus, in wheat, an increase in root mass of 50–90% relative to the control was observed with SNP treatment at different concentrations. A slightly smaller but quite significant increase in root weight was observed in triticale when seeds were treated with SNP, with an increase of 15-45% at different concentrations. Shoot weight in wheat seedlings was significantly increased by SNP concentrations of 100 and 500 µM (Figure 1B).

In triticale, a significant effect on shoot weight increase was only observed for the 100 µM concentration. In general, seedling weight in wheat increased as a result of SNP priming at all concentrations used, while in triticale this effect was significant for SNP concentrations of 20 and 100 µM (Figure 1B).

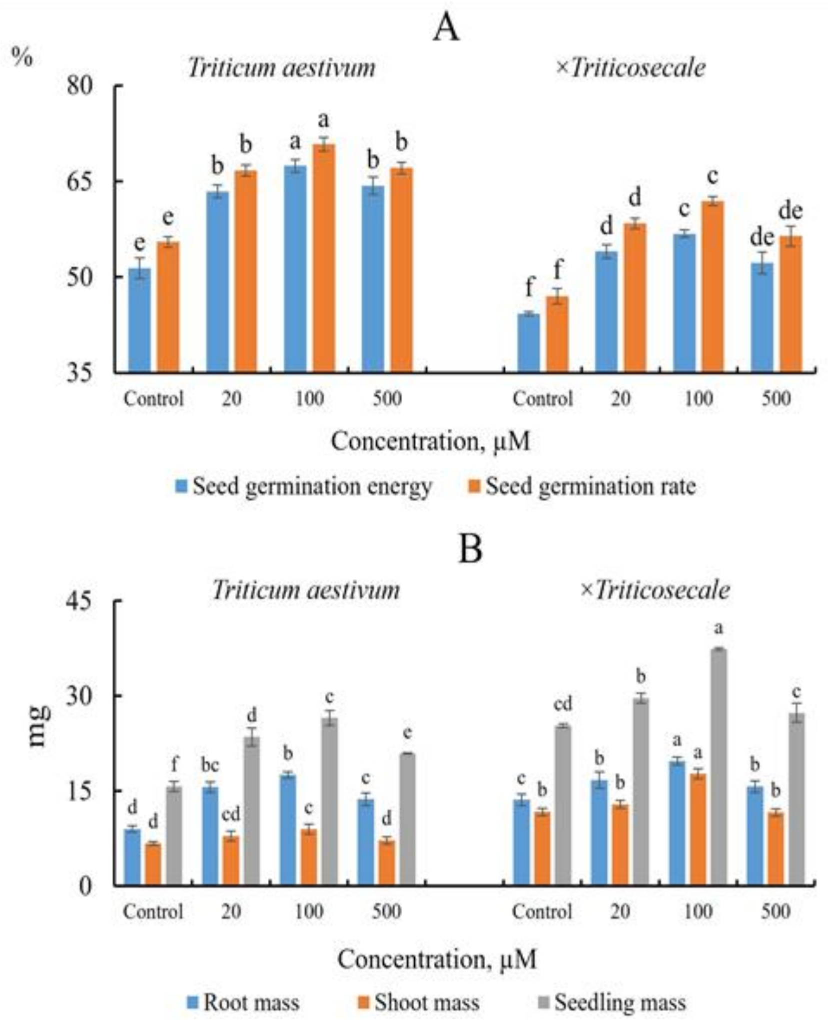

Figure 1. Concentration dependence of the effect of SNP priming on germination energy, seed germination rates (A) and organ biomass (B) of wheat and triticale seedlings

The specificity of the action of SNP as a nitric oxide donor rather than a complex compound is usually confirmed by the use of the so-called 'exhausted' SNP solution, which, as mentioned in the Materials and Methods section, is obtained by decomposition of this salt in the light (Mur *et al*., 2013). As our results showed, the 'exhausted' SNP at a concentration corresponding to the most effective concentration of freshly prepared SNP (100 µM) had no significant effect on any of the parameters characterising seed germination and seedling growth in wheat (Figure 2, Table 1). In triticale, no significant effect of the 'exhausted' SNP on germination energy and germination indices was found. At the same time, when seeds were treated with the 'exhausted' SNP, a slight increase in root weight and, as a consequence, in total seedling weight was observed (Table 1). Thus, we can speak of a small non-specific growth stimulating effect of the 'exhausted' SNP when triticale seeds were treated with it. However, these effects were much smaller compared to the effects of freshly prepared SNP.

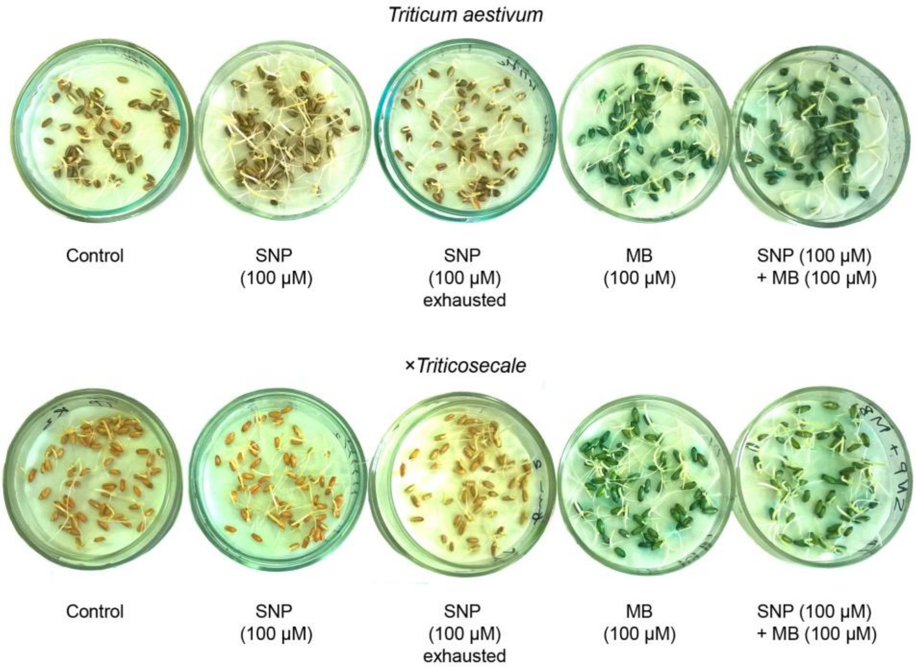

Figure 2. Condition of wheat and triticale seedlings when seeds were treated with SNP, 'exhausted' SNP and the NO scavenger methylene blue (MB)

Another way of demonstrating the specificity of the effect of SNP as an NO donor is to use it in combination with nitric oxide scavengers. In our experiments, treatment of wheat seeds with SNP in combination with methylene blue completely abolished the effect of the nitric oxide donor on all the indicators of seed germination and seedling growth studied (Table 1).

The combined effect of SNP and methylene blue on triticale seeds did not show the effect of NO donor on germination energy, although the germination index in the variant with the combination of SNP and methylene blue was slightly higher than in the control, but significantly lower than in the variant with priming seeds with SNP alone (Table 1). Seed treatment with methylene blue also completely eliminated the effect of SNP on seedling growth parameters.

It should be noted that in the variant with priming of wheat seeds with methylene blue only, a significant decrease in germination energy and germination was observed, although the growth of seedling organs in this variant did not differ from the control. In triticale under the influence of seed priming with methylene blue, a significant decrease in seed germination and root biomass was recorded (Table 1). In general, treatment of wheat and triticale seeds with methylene blue slightly worsened their germination, indicating the role of endogenous nitric oxide in the germination of seeds of these cereal species.

Table 1. Modification of the SNP effect on seed germination and morphometric indices of seedlings with its pre-light treatment and combination with methylene blue (MB)

Variant	Germination energy, %	Seed germination rates, %	Root weight, mg	Shoot weight, mg	Seedling weight, mg
Triticum aestivum L.					
Control	49.7±1.0 cd	53.6±1.2 c	10.0±0.6 f	6.7±0.3 d	16.7±0.8 de
SNP 100 μM	64.5±1.1 a	69.8±1.2 a	16.4±0.9 b	9.3±0.4 c	25.7±0.8 bc
SNP 100 μM 'exhausted'	52.2±0.4 c	55.5±1.4 c	10.2±0.9 f	7.0±0.7 d	17.2±0.3 de
SNP (100 μM) + MB (100 μM)	44.7±1.5 de	46.7±1.1 de	10.0±0.3 f	7.7±0.7 d	17.7±0.4 d
MB (100 μM)	43.2±1.5 e	46.3±0.9 de	8.6±0.4 f	7.1±0.3 d	15.7±0.1 e
×*Triticosecale*					
Control	45.7±1.7 de	48.4±0.7 d	12.5±0.5 d	12.0±0.2 b	24.5±0.4 c
SNP 100 μM	57.7±0.7 b	61.2±0.8 b	18.7±0.7 a	16.7±1.0 a	35.4±1.7 a
SNP 100 μM 'exhausted'	44.3± 0.9 e	47.7±1.5 d	14.7±0.6 c	12.7±0.8 b	27.4±0.2 b
SNP (100 μM) + MB (100 μM)	47.3±1.0 d	53.1±1.6 c	11.8±0.6 de	12.8±0.9 b	24.7±0.3 c
MB (100 μM)	42.2±1.1 e	44.6±1.6 e	13.1±0.3 d	10.7±0.7 c	23.8±0.4 c

Amooaghaie and Nikzad (2013) showed that methylene blue eliminated the enhancement of tomato seed germination induced by SNP treatment under low temperature conditions. Another NO scavenger, cPTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), inhibited seed germination of the woody plant Paulownia elongata, which is characterised by physiological dormancy (Liu *et al.*, 2019). Thus, our data on the retardation of germination of aged wheat and triticale seeds in the presence of methylene blue and its abolition of the positive effect of SNP on seed germination are consistent with data on the role of endogenous and exogenous NO in the interruption of seed dormancy obtained for other plant species.

Effect of SNP on ROS generation and MDA levels in wheat and triticale seedlings

When studying the effect of nitric oxide donor on indicators characterising the level of oxidative stress during seed germination, it was used at the concentration that had the most significant effect on seed germination and seedling growth, 100 μM. Under the influence of SNP, a significant decrease in the generation of superoxide anion radicals was observed in wheat seedlings and even more significantly in triticale (Figure 3A). Hydrogen peroxide content was also reduced under the influence of SNP seed priming in shoots of seedlings of both species, but this effect was more pronounced in triticale seedlings (Figure 3B). The amount of the LPO product MDA in SNP-primed variants decreased in both cereal species, but more significantly in wheat (Figure 3C).

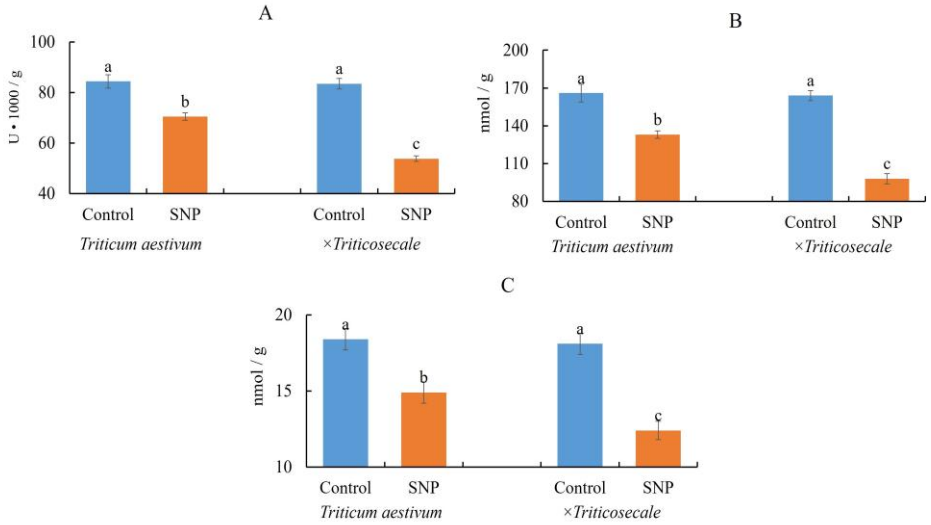

Figure 3. Effect of seed SNP priming on superoxide anion radical generation (A), hydrogen peroxide (B) and MDA (C) content in wheat and triticale seedlings

In general, the treatment of wheat and triticale seeds resulted in a reduction in all the studied parameters that characterise the level of oxidative stress. In this context, the influence of SNP on the functioning of the antioxidant system was studied in subsequent experiments.

Effect of SNP seed priming on antioxidant enzyme activity in wheat and triticale seedlings

SOD activity was higher in wheat seedlings (Figure 4A). At the same time, SNP treatment caused its decrease. In triticale seedlings, this index also decreased under the influence of SNP. At the same time, there is evidence in the literature about the positive modulation of SOD activity by nitric oxide donors due to the corresponding post-translational modification (Sougrakpam *et al*., 2018; Pereira *et al.*, 2022). It should be noted that in our experiments, the decrease in SOD activity in the nitric oxide donor seed treatment variant in seedlings of both cereal species was accompanied by a concomitant decrease in superoxide anion radical generation. A possible explanation for this apparently paradoxical effect could be suppression of NADPH oxidase activity by nitric oxide due to post-translational modification of the enzyme (Yun *et al*., 2011). However, further studies are needed to clarify the interpretation of these results.

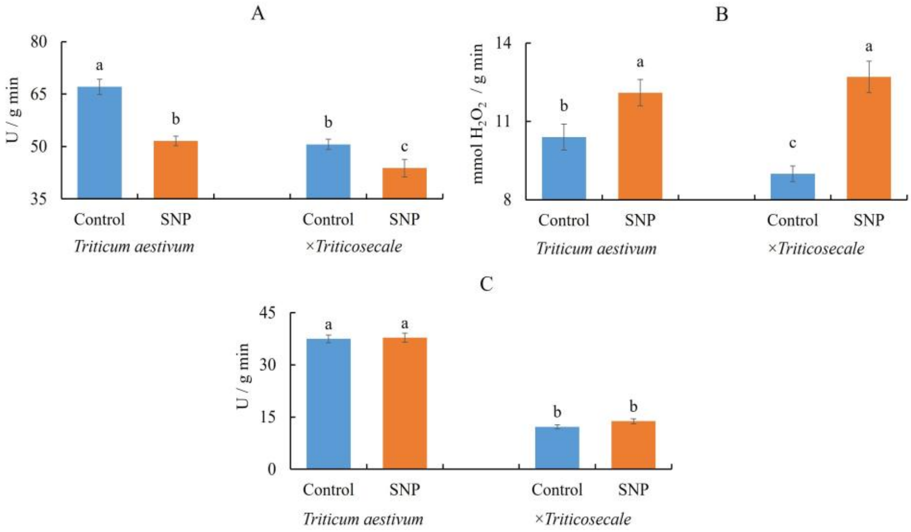

Figure 4. Effect of seed SNP priming on SOD (A), catalase (B) and guaiacol peroxidase (C) activity in wheat and triticale seedlings

SNP priming of seeds of both cereal species resulted in an increase in catalase activity (Figure 4B). This effect is consistent with data obtained when SNP priming of aged sunflower seeds was studied (Pereira *et al.*, 2022). SNP priming of peanut seeds also promoted catalase activity during germination under osmotic stress conditions (Sepehri and Rouhi, 2016). The effects of increased catalase activity under the influence of NO donor have been attributed to S-nitrosylation of specific cysteine residues (Sougrakpam *et al.*, 2018). However, specific studies are also needed to assess the contribution of this mechanism to the regulation of catalase activity under specific experimental conditions.

The activity of guaiacol peroxidase in the control variant was much lower in triticale seedlings than in wheat seedlings (Figure 4C). As shown by studies with other varieties of these cereals, low guaiacol peroxidase activity is a species characteristic of triticale (Kolupaev *et al.*, 2022b). However, under the conditions of our experiments, SNP had no effect on enzyme activity in seedlings of either cereal species. It is known that non-specific peroxidase (guaiacol peroxidase) can not only neutralise ROS, but also participate in ROS generation, i.e. exhibit pro-oxidant effects (Kolupaev and Karpets, 2014). Therefore, an increase in peroxidase activity is not always a sign of activation of antioxidant processes. At the same time, catalase, whose activity was increased by SNP seed treatment, effectively fulfils the functions of neutralising high concentrations of H_2O_2 (Das and Roychoudhury, 2014). In this context, the increase in its activity in the absence of changes in the activity of guaiacol peroxidase in seedlings from SNP-primed seeds can be considered as the reason for the marked decrease in their hydrogen peroxide content.

Effect of SNP seed treatment on the content of secondary metabolites in seedlings

It is established that secondary metabolites, namely phenolic and polyphenolic (flavonoid) compounds, play a pivotal role in the functioning of the antioxidant system of cereals (Kolupaev *et al.*, 2023c). In this context, we investigated the effect of SNP on the content of phenolics and anthocyanins in shoots of wheat and triticale seedlings.

SNP priming of wheat seeds caused an increase in the total phenolic content (Figure 5A), while the anthocyanin content did not change (Figure 5B). A different pattern was observed in triticale seedlings derived from SNP-primed seeds. They showed a significant increase in the content of anthocyanins with no significant changes in the total content of phenolic compounds (Figure 5). It should be noted that the high basal content of anthocyanins and its increase in response to adverse factors is a species characteristic of triticale and distinguishes it from most wheat varieties (Kolupaev *et al*., 2020b; 2022b). At the same time, as mentioned above, the blue-grained wheat variety Scorpion used in our work is characterised by a high content of phenolic compounds in general (Martinek *et al.*, 2013). Thus, their amount in etiolated seedlings was the same as in triticale seedlings (Figure 5). It can be assumed that the increase in phenolic compounds in wheat and flavonoids (anthocyanins) in triticale during seed priming with a nitric oxide donor contributed to the reduction of superoxide radical generation (Figure 3A) and possibly other radical ROS during seed germination. Secondary metabolites, and anthocyanins in particular, are known to be effective in neutralising ROS, primarily radical forms, including the superoxide anion radical (Neill and Gould, 2003).

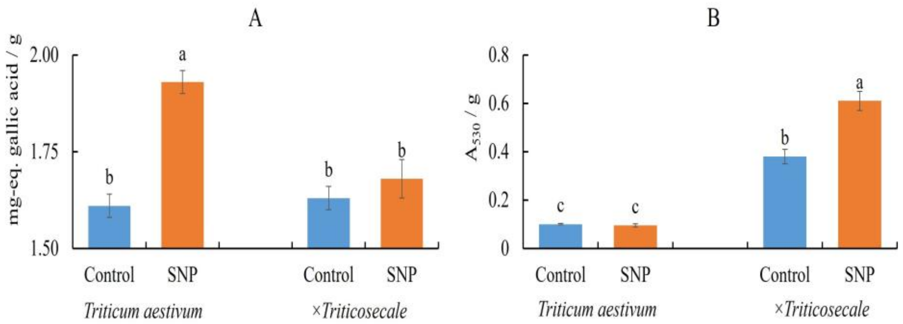

Figure 5. Effect of seed SNP priming on the content of phenolic compounds (A) and anthocyanins (B) in shoots of wheat and triticale seedlings

Possible relationship between the effect of nitric oxide donor on redox homeostasis and seed germination

In general, the priming of old wheat and triticale seeds, which promoted their germination and seedling growth, significantly modified the studied indicators of the pro/antioxidant balance (Figure 6). The levels of all markers of oxidative stress decreased in the seedlings of both cereal species: superoxide anion radical generation, hydrogen peroxide and MDA content. In the context of reduced $O_2^{\bullet-}$ formation under the action of NO donor, SOD activity decreased in both species. At the same time, the activity of the key hydrogen peroxide decomposing enzyme, catalase, increased in both species when seeds were treated with SNP. In the absence of changes in guaiacol peroxidase activity, it can be assumed that catalase was the major contributor to the regulation of hydrogen peroxide levels in seedlings. Secondary metabolites, such as various phenolic compounds in wheat and anthocyanins in triticale, also appeared to contribute to the regulation of the levels of different ROS during SNP treatment (Figure 6).

Undoubtedly, the above explanation of the effect of nitric oxide donors on ROS generation and the state of the antioxidant system in seedlings of the studied cereals is very schematic and simplified. According to modern models, nitric oxide can exert a complex influence on the pro/antioxidant balance in plant cells. It involves post-translational modification of proteins (including directly antioxidant enzyme molecules), which can lead to both increased and decreased activity, depending on the type of modification (S-nitrosation or tyrosine nitration) (Aroca *et al*., 2015; Correa-Aragunde *et al*., 2015; Corpas *et al.*, 2019). Nitric oxide can also modulate the activity of pro-oxidant enzymes such as NADPH oxidase (Yun *et al.*, 2011). In addition, NO affects enzymes responsible for maintaining the pool of reducing agents (Aroca *et al.*, 2015; Corpas *et al.*, 2021), which may also alter the pro/antioxidant balance in cells. Finally, through its involvement in the regulatory network, nitric oxide, together with ROS, can influence gene expression of antioxidant enzymes (Kolupaev *et al*., 2023a).

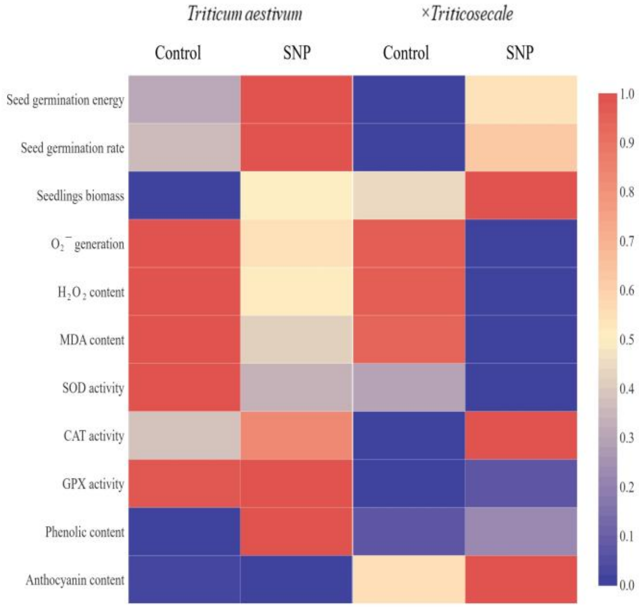

Figure 6. Heat map of changes in growth parameters and antioxidant system of wheat and triticale seedlings under the influence of 100 μM SNP. All values are normalised from 0 to 1

However, it is difficult to interpret the mechanisms of nitric oxide influence on the antioxidant system of seedlings after seed priming because it is not clear how long the effect of SNP lasts after its treatment. At the same time, the lack of effect of SNP on seed germination and seedling growth after light treatment or when used in combination with the nitric oxide scavenger methylene blue indicates the key role of NO in the physiological effects of SNP (Table 1).

CONCLUSIONS

The results obtained indicate that priming aged wheat and triticale seeds with the nitric oxide donor SNP can be an effective tool for increasing their germination and improving seedling growth. At the same time, an important component of the SNP effect is the alleviation of oxidative stress effects during germination of aged cereal seeds (reduced generation of superoxide anion radical and hydrogen peroxide, decreased malondialdehyde content). Seed treatment with SNP modulated the activity of antioxidant enzymes SOD and catalase in cereal seedlings, and also enhanced the accumulation of secondary metabolites with antioxidant properties.

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BIO-FUNCTIONALITY OF BRINED CHEESES: COMPARISON BETWEEN CURD COOKED AND UNCOOKED TYPES

SUMMARY

The cooking temperature of cheese affects the degree and pattern of proteolysis due to the residual activity of chymosin, leading to variations between different cheese varieties. The main objective of this research is to investigate the chemical and biochemical changes in brined cheeses produced with and without cooking, in order to control and enhance cheese quality. Brined cheeses made with ewe's milk combined with different technologies of curd treatment (cooked-C vs uncooked -N), were studied for their total free amino acid (TFAA) content, water-soluble nitrogen (WSN), 12% trichloroacetic acid-soluble nitrogen (TCA-SN) and angiotensin-converting enzyme-inhibition activity (ACE-i). (N) cheeses had the highest pH, fat, and salt value than (C) cheese. Also, the higher TFAA mg Leu/kg, DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2-azinobis, 3-ethylenebenzothiazoline-6-sulfonic acid) were conducted in the uncooked cheese. Cooked curd cheese was the most active with an ACE-i activity of 93.1%. On the contrary, the same cheese showed the lowest antioxidant activity (88.1%). The RP-HPLC profiles show that the formation rate of smaller peptides was increased in cheese made without heat treatment than in heat treated one probably due to the heat deactivation process of proteolytic enzymes. Maillard reaction during cooked (C) cheeses has implicated that volatile formation is caused through reactions in higher temperature treatment.

Keywords: Cooked cheese, proteolysis, enzyme-inhibition activity (ACE-i), peptide profile, volatiles

INTRODUCTION

The city of Tetovo lies in the Polog region, North Macedonia, at the base of the Sharr Mountain on a surface of 1,080 km^2 at an altitude of 460-500 m, in the course of the river Pena. Polog's lowland lies in the southeast-northwest direction, at a distance of 55 km and a width of 8-10 km. It has a semi-continental climate, with warm and relatively humid summers, cold and snowy winters, and autumn falls are frequent rainfall. Since it is surrounded by mountains on all sides and due to the appearance of hot and cold air streams, in the Polog area comes the

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emergence of the inversion process. Natural conditions, such as climate, topography, and geological composition, have allowed numerous springs of running water to emerge on the outskirts of Tetovo. This makes Tetovo one of the few towns in Macedonia with access to drinking water, as well as water for industrial and irrigation purposes (Abdii and Xhulaj, 2016). In the Tetovo district, the sheep population is approximately 20035, which includes 17251 Sharplaninka, 1,224 Ovčepolka, 201 Karakaçnanska, 463 Witendberg, 6 Crnagllava, and 6 Avasi, among others. The goat population consists of 1,695 total goats, including 327 Alpina, 75 Saanen, 660 Balkan, 218 Alpine hybrids, 13 hybrids of Saanen species, and 390 others. The local dairy community comprises 30 cheese producers from the Sharr Mountain, Dry Mountain, and Zheden Mountain regions, with the grazing and milking season beginning between June 1st and June 10th (SSO, 2015-2020).White cheese is a brined variety of cheese with either soft or semihard texture, white colored and with close texture, pickled flavour commonly made from cow milk, sheep milk and ripened in brine for 60 days. Traditionally, has been produced by farmers using raw milk and traditional techniques using only elementary equipment. According to North Macedonian Regulation, there are following groups of cheeses: cheeses with ripening, cheeses with molds, cheeses in brine, cheeses with steamed dough and cheeses without ripening (Official Gazette of Republic of Macedonia, 2011). Cheeses in brine are cheeses in which ripening and storage until consumption takes place in brine. It is produced from raw milk and also pasteurized milk in industrial conditions in the Balkan region (Sulejmani and Selimi, 2022). The composition type of cheeses varies by many factors such as milk composition, pre-treatment and ripening requirements. Protein hydrolysis is considered to involve the polypeptide formation to enhance the biological value or functional properties, enhance flavor quality, and provide a way for new protein synthesis (Sulejmani and Hayaloglu, 2020). Pešić *et al.* (2014) conducted that treatment for 10 min at 90 ° C incorporates whey proteins (more than 95%) as complexes of all three caseins (κ-, b- and aS2). The presence of whey proteins significantly enhances the nutritional value of cheese due to their excellent amino acid composition. Traditional brined cheeses from North Macedonia are highly valued products known for their intense flavors. However, there is a lack of comprehensive data on comparative studies regarding the impact of cheese curd heat treatment on their bioactivity. This investigation aims to provide insights into the proteolysis status and volatile compounds present in brined cheese produced under various curd conditions. The quality of cheese can be greatly influenced by the heat treatment of the curd. Therefore, this study focuses on evaluating the technological processes involved in curd treatment for brined cheese production and how these processes affect the biofunctional properties of the cheese.

MATERIAL AND METHODS

The study was conducted in a local sheep farm in Tearce village (42°07`50.00"N, 21°05`32.20"E) located 12 km to the northeast of Tetovo with an altitude of 500 meters, northwestern part North Macedonia. The sheep graze in the field grass and polygonal plants of the meadows. Geographical region, milking season and animal feed influence the variable quality of milk used to produce these traditional cheeses.

Figure 1. Map of the traditional brined cheese production area (arrows)

Fresh raw milk from 180 sheep was used during milking in the morning collecting 85 litres of milk. From 35 litres of milk, 7 kg of (N, uncooked) cheese and from 50 liters of milk, 12 kg of (C, cooked) cheese was produced (Fig 2). The dry matter, protein, salt, fat and total free amino acid (TFAA) content in the brined cheeses were determined according to Ardo and Polychroniadou (1999). The water-soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble nitrogen (TCA-SN) as % of total nitrogen of the cheeses were determined by the methods as described by Kuchroo and Fox (1982). Total free amino acids (TFAAs) were determined on the water-soluble fraction of the cheeses by the cadmium-ninhydrin method as described in Folkertsma and Fox (1992). pH meter with combined electrode (Orion, USA) was used for determination of the pH of the cheeses. Reversed-phase high-performance liquid chromatography (RP-HPLC) and static solid-phase microextraction (SPME) method using a gas chromatography mass spectrometry (GC-MS) system (Shimadzu, Kyoto, Japan) were used for analysis of peptide profile and volatiles in cheeses, respectively (Sulejmani and Hayaloglu, 2018).

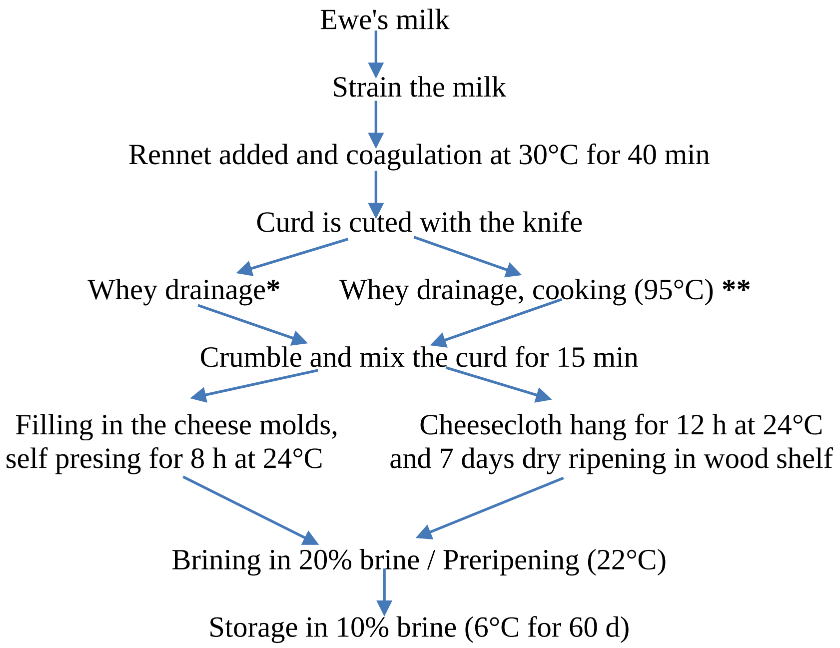

Figure 2. Technological process of production cooked (C)** and uncooked (N)* brined cheeses

To evaluate DPPH free radical scavenging ability (RSA), ABTS•+ radical scavenging activity, and ACE inhibitory activity of salted cheeses, the methods of Brand-Williams *et al.* (1995), Re *et al.* (1999) and Sahingil *et al.* (2014) were used, respectively (Fig 3 a,b,c). Samples were chemically analysed in triplicate or more on the 1st week of ripening (fresh).

For the statistical analyses in this study, SPSS (SPSS Inc., USA) was used. All results are expressed as mean ± standard deviation. For statistical differences between cheeses (C) and (N) in the type of salted cheese, the independent T-test was used at the level of significance of differences ($P < 0.05$).

RESULTS AND DISCUSSION

The chemical compositions of the brined cheese samples are given in Table 1. The pH value affects the growth of microorganisms and the activity of enzymes. The higher pH value was in (N) cheese (5.16), than in (C) cheese (5.12) ($P<0.05$). The higher pH in (N) brined cheeses occurs due to the intensive proteolysis or proteins breakdown and formation of ammonia and metabolism of lactic acid (Sulejmani *et al.*, 2014). The protein of the (C) cheese was higher than uncooked (N) cheese ($P<0.05$). The content of protein in cheese depends on the amount of casein in raw milk and cheese production techniques. The results showed that the (N) cheeses have the lower dry matter and fat values compared to the cooked brined cheese ($P<0.05$). The salt content of the brined cheeses is similarly with the most varieties of brined cheese (Bintsis and Papademas, 2018).

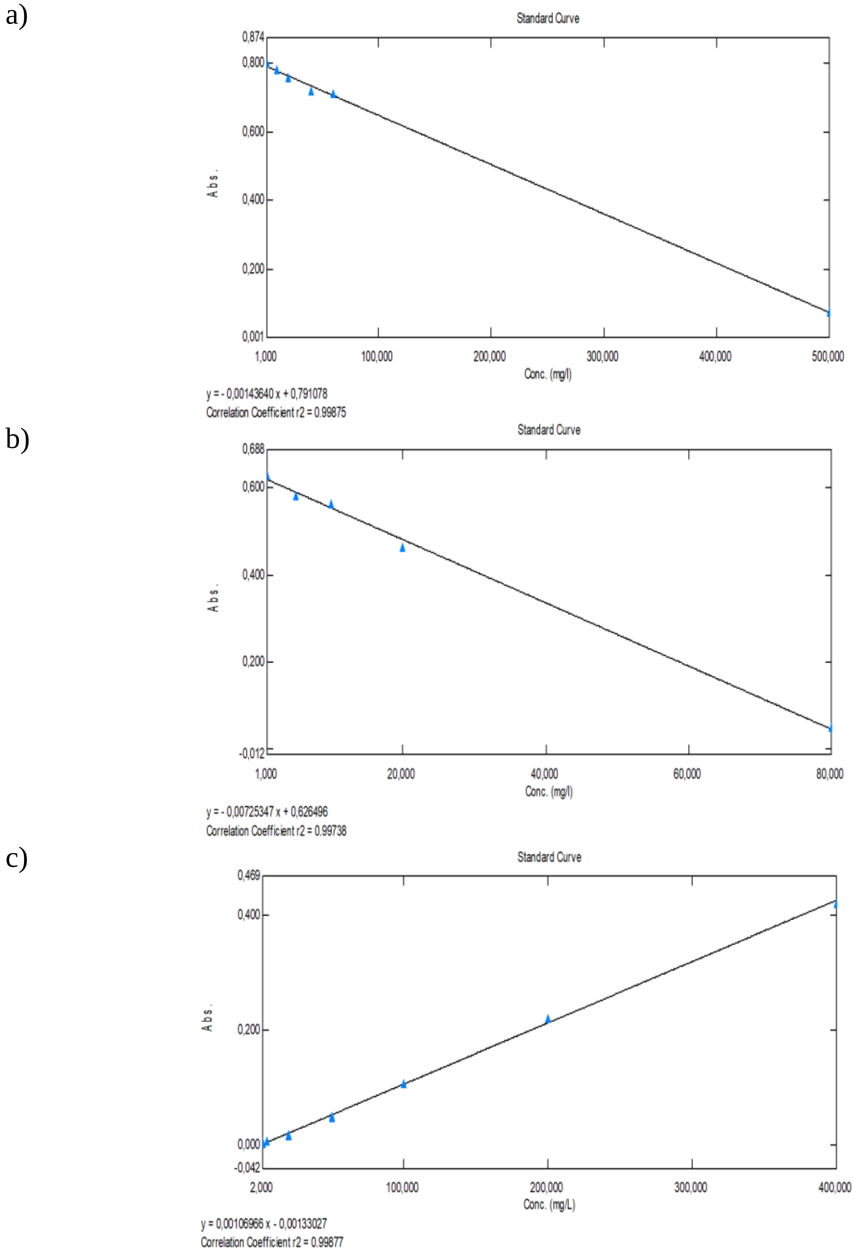

Figure 3. Calibration curve of the DPPH (a), ABTS (b) and TFAA (b) solutions

The dry matter of the brined cheeses are higher than that reported for fresh Turkish Beyaz cheese (Turkish Food Codex: 2015/6). The values for proteolysis respectively soluble nitrogen (WSN), expressed as % of TN, total free aminoacids, and peptide profile in brined cheeses made from cooked and uncooked curd are shown in Table 1 and Figure 4. The action of milk and rennet proteases is expressed through indicators respectively parameters of soluble nitrogen compounds (SN) in cheese as an indicator of the extent of proteolysis (Kalit *et al.*, 2005).

Table 1. Physical-chemical and bioactivity properties of fresh brined cheeses

Parameters [a]	**(C)**	**(N)**	*ANOVA**
pH	5.12±0.02	5.16±0.01	*s*
Dry matter	46.52±0.20	45.00±0.32	*s*
Protein	22.84±0.21	16.16±0.33	*s*
Fat	22.25±0.22	26.75±0.34	*s*
Salt	1.76±0.23	2.44±0.35	*s*
WSN/TN (% of TN)	1.7±0.09	4.09±0.37	*s*
TCA/TN (% of TN)	0.72±0.05	0.53±0.31	*s*
TFAA mg Leu/kg	139.9±0.14	173.80±0.16	*s*
%ACE-i	93.05±0.02	82.75±0.05	*s*
DPPH	88.04±0.66	98.29±0.650	*s*
ABTS	17.09±0.50	18.31±0.49	*s*

[a] *Abbreviations: Values are means ± SD; C- cooked, N- uncooked. S-differ significantly (P<0.05). WSN, water-soluble nitrogen TCA;12% trichloroacetic acid-solublenitrogen; TFAA, total free amino acid; angiotensin converting enzyme-inhibition activity (ACE-i).DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azinobis, 3-ethylenebenzothiazoline-6-sulfonic acid)*

The highest soluble nitrogen (SN) was found in cheese produced without curd cooking. Also enzymes of non-starter cultures act by degrading high and medium molecular weight peptides (Sulejmani and Hayaloglu, 2017). Uncooked cheese had significantly higher antioxidant activity probably due to higher fat content and presence of vitamin E as antioxidant compounds (McSweeney and Sousa, 2000).

In the area of 12–28 min in the chromatogram of (N) cheeses were an increase in peaks of peptides. Whereas, between 32 and 36 min, the peak in (C) cheeses were higher than (N) cheeses. Corresponded peptides eluted on 40–64 min were noted to be higher in (N) cheeses. Changes in production processes (thermal treatment of coagulum), caused changes in the activity of the remaining coagulant and changed the extent of proteolysis. However, degradation of as1- and b-caseins were noticeable lower in (C) cheese than in (N) cheeses due to the high temperature used in the manufacturing of cooked cheeses that indicates lower retention of residual coagulant (Sulejmani and Hayaloglu *et al.*, 2016). At retention times < 15 min, small hydrophilic peptides were detectable in both cheeses. Large hydrophobic peptides seemed between 60–78 min were higher in the (N) cheese which may correlate with the cheese bitterness. The pH also affects the reduction of the aromatic activity and the instability of the anions of the dissociated fatty acids (Alewijn, 2006). The wood used in the drying process of thermally treated cheese contains free extractable compounds of low molecular weight in its porous structure including volatile and non-volatile organic compounds (Licitra *et al*., 2018).

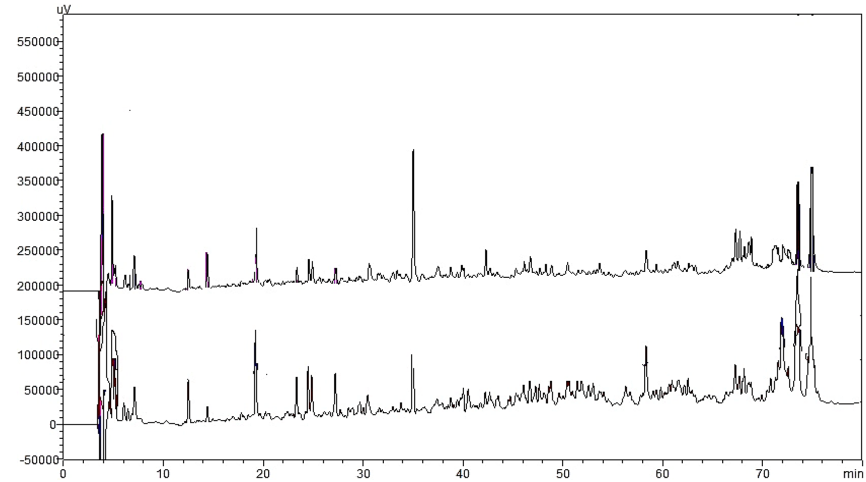

Figure 4. Peptide profile of brined cheeses from cooked (C) and uncooked (N) curd

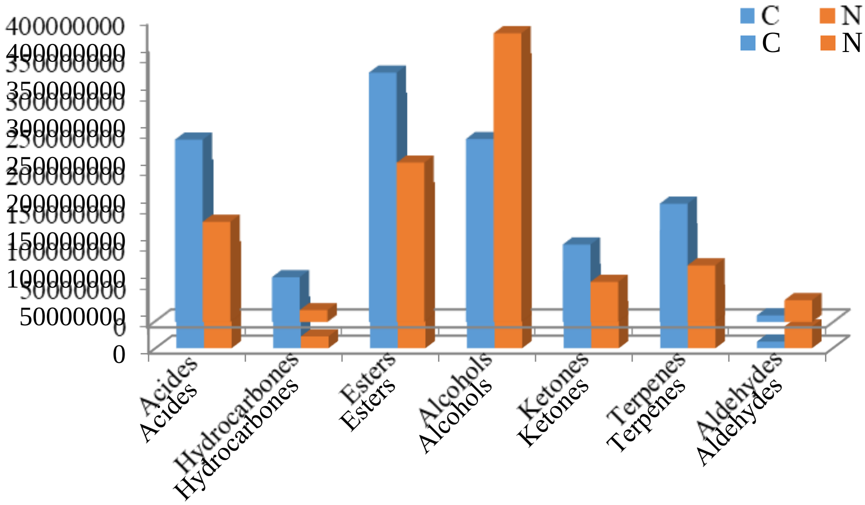

Figure 5. Relationship between average area values of volatile compounds group in cooked (C) and uncooked (N) brined cheeses identified bu GC-MS analysis

In the volatile fraction of the both brined cheeses, the volatile components consisted of 8 ketones, 8 acids, and 17 esters. However cooked brined cheeses had higher numbers of terpenes (12) and hydrocarbons (13) while uncooked ones had (18) alcohols and (9) aldehydes (Table 3). Cooked cheese is characterized by a higher amount of volatile substances, especially organic acid which can also promote ketone formation (Forss, 1979) (Fig 5; Table 2). The similar results were conducted during characterization of flavor characteristics of cooked cheese were organic acid formation are likely to be due to thermally induced reactions (Sullivan *et al.*, 2023).

Reactions caused by heat treatment including amino acids and their increased catabolism are enabled by cooking through the continuous network of casein (Banks *et al*., 2001). It is highlighted from previous data that dipeptides are sensitive to the Maillard reaction and act as precursors of volatile aromatic compounds (Van Lancker *et al*., 2010).

Table 2. Types of volatile compounds isolated from two kinds of brined cheese

Compounds	**Cooked**	**Uncooked**
Terpenes	**12**	**5**
Esters	**17**	**17**
Acides	**8**	**8**
Alcohols	**17**	**18**
Ketones	**8**	**8**
Aldehydes	**3**	**9**
Hydrocarbones	**13**	**9**
Total	**78**	**74**

Thermally induced reactions, such as the Maillard reaction and caramelization, affect the concentration of volatile compounds during cooking. These reactions also influence the flavor of cooked cheese by contributing to the formation of specific non-volatile substances within the cheese.

CONCLUSIONS

The activity of enzymes and biochemical processes in brined cheese produced with cooked curds was significantly affected, leading to differences in chemical composition, proteolysis, and volatile compounds. The results of this study indicated that uncooked curds enhanced the production of alcohols and aldehyde volatiles in cheese, with their concentrations being higher in cheeses made from uncooked (N) curds than in those made from cooked (C) curds. These findings suggest that heat-induced changes to the cheese curd are the primary cause of the differences observed in the levels of ACE-inhibiting peptides in the cheeses. The peptide profile revealed a higher formation of smaller peptides in cheese made without heat treatment compared to that made with cooking. The cooking process, which involves high-temperature reactions such as the Maillard reaction, significantly impacts the production of aromatic volatile compounds.

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IMPLEMENTING NEW PREDICTIVE FUNCTIONAL MODEL FOR MILK FAT VALUE IN MACEDONIAN WHITE BRINED CHEESE PRODUCTION

SUMMARY

In the production of Macedonian white-brined cheese, milk fat content is a crucial determinant of the final product's quality, texture, and taste. Accurate prediction and management of milk fat levels during cheese production are essential for maintaining consistency, optimizing yield, and ensuring consumer satisfaction. This study presents the implementation of a new predictive functional model specifically designed to estimate milk fat value in the production of Macedonian white-brined cheese. The model integrates various factors, such as the initial composition of raw milk, processing conditions, and key technological parameters that influence fat retention and distribution throughout the cheese making process. By using a combination of statistical analysis and machine learning techniques, the model enables a more precise and real-time prediction of milk fat content, addressing challenges related to seasonal variations in milk composition and other unpredictable factors in dairy production. Data from local dairies were used to validate the model's performance, and results demonstrate its accuracy in predicting milk fat values with a high degree of reliability.

The study's most significant findings demonstrate the variation and trends in milk fat content across four cheese variants (A, B, C, and D) during the ripening process. The results show that Variant D consistently maintained the highest milk fat values throughout the ripening period, with significant differences ($p<0.05$) compared to the other variants. By the 60th day of ripening, Variant D had a milk fat content of 25.41±0.02%, which was 2.3% higher than Variant C, the variant with the lowest fat content.

The predictive functional model achieved high R^2 values for all variants, ranging from 0.9673 to 0.9997, indicating its robustness in estimating milk fat

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dynamics. Variant A achieved an R^2 of 0.9997, demonstrating near-perfect alignment between the model's predictions and experimental data.

The implementation of this model has the potential to streamline production processes by reducing the need for frequent laboratory analyses and allowing producers to make real-time adjustments during manufacturing. Furthermore, it contributes to enhanced product standardization, better resource management, and overall improvement in cheese quality. This predictive model offers a novel tool for dairy producers in N. Macedonia and beyond, aiming to improve the efficiency and quality control in white-brined cheese production.

Keywords: white brined cheese, milk fat, quality control, functional model

INTRODUCTION

White-brined cheese is a popular cheese type in many parts of the world, which has been enjoyed for centuries, particularly in Mediterranean cuisine. It belongs to the group of cheeses that ferment in brine solution in anaerobic conditions. This product is characterized with acid salty flavor, no rind, usually white color, but sometimes with yellowish tint, anaerobic brine fermented in plastic cans and pieces which are usually in form of cubes with dimensions 10x10x10 cm, (Velevski, 2015). According to the Codex Alimentarius (Codex Stan 208-1999), white brined cheese belongs to the category of "Cheese in Brine." This category is defined as follows: "Cheese in Brine" refers to cheeses that are ripened and stored in a brine solution, which contributes to their characteristic texture and flavor. These cheeses typically have no or only a very thin rind and may range in texture from soft to semi-hard.

The significance of milk fat in cheese-making is identified by the findings of numerous studies in dairy science and food chemistry. Milk fat remains a critical factor in cheese-making, particularly in influencing flavor, texture, and overall sensory quality of the end product, (McSweeney *et al.*, 2013). New studies, such as those by Franceschi *et al.*, (2024), emphasize the importance of fat, casein, and protein content in the quality of dairy products, and their impact on coagulation properties, which are crucial in determining cheese yield and texture.

Milk fat is one of the most dynamic and variable components found in various types of milk. Maintaining the required level of milk fat value is crucial in all varieties of cheeses, and this is achieved through the milk standardization process. Numerous factors play significant role in determining the content of milk fat in white cheese, with the most prominent influences being the composition of the milk, particularly the casein-to-fat ratio, and also the technological processes used in cheese production, (Bojanić-Rašović *et al.*, 2013).

Research by Haenlein and Wendorff (2006) highlights the multi-faceted contributions of milk fat to cheese quality, emphasizing its role in flavor development, enhancement of taste, and texture modification. Furthermore, investigations by Fox *et al.*, (1998) explain the intricate biochemical processes through which milk fat constituents interact with microbial enzymes during cheese ripening, thereby influencing flavor maturation and textural evolution. The influence of milk fat on white cheese quality is significant and multifaceted. Milk fat contributes to the flavor profile of cheese. Higher fat content often results in a richer, creamier taste. In white cheese varieties fat content can enhance the buttery or nutty notes, contributing to a more enjoyable eating experience, (Waldron *et al.*, 2020). The fat content affects the rheology and texture of the cheese, but produced a limited effect on taste and aroma. Milk fat value can influence the appearance of white cheese. Higher fat content often results in a richer color and a more attractive appearance, as a significant factor in consumer appeal (Guinee and McSweeney, 2006).

The numerous compounds in cheese aroma are derived mainly from three major metabolic pathways occurring during cheese ripening: catabolism of lactose, lactate, and citrate, lipid and protein catabolism. Milk fat contains volatile compounds that contribute to the aroma of cheese. Higher fat content can lead to a more complex and pronounced aroma, which can enhance the overall sensory experience of consuming white cheeses, (Arias-Roth *et al.*, 2022). Milk fat is a source of energy and essential fatty acids. While higher fat content contributes to the calorie and fat content of cheese, it also provides a richer source of nutrients. The concentration of milk fat in cheese holds significant influence over crucial aspects such as taste, aroma, consistency, microstructure, and the biochemical and rheological characteristics of the final product. The complex interactions between these variables highlight how crucial it is to closely monitor and regulate the amount of milk fat during the cheese-making process (Guinee *et al*., 2000).

Fox *et al.*, (2017) further emphasizes the nutritional significance of milk fat in dairy products, stating, 'The composition and properties of milk fat secure a high nutritional and biological value to the products. The synergy between milk fat and proteins contributes not only to the taste and texture of cheese but also to its overall nutritional profile.

The primary aim of this research is to design and implement an innovative predictive functional model for estimating milk fat content in the production of Macedonian white-brined cheese. This study seeks to address challenges associated with milk fat variability, ensuring a consistent and high-quality final product.

The main objectives are as follows: to analyze the dynamics of milk fat content during the ripening process of cheese produced under different production conditions and to develop and validate a predictive functional model that accurately estimates milk fat levels based on critical factors, including initial milk composition, processing parameters, and technological conditions.

The significance of this research lies in the practical applications of the predictive model. It serves as:

•A tool for quality control: Helping producers monitor and maintain consistent cheese quality.

•A process optimization resource: Reducing dependency on frequent and costly laboratory analyses by enabling real-time adjustments during production.

•A driver for standardization: Facilitating greater consistency and uniformity in white-brined cheese production.

•A broader innovation: Offering insights and methodologies that can be applied to the wider dairy industry, supporting resource efficiency, sustainability, and the development of innovative dairy products.

MATERIAL AND METHODS

Sample Collection and Transportation. This study utilized four variants of Macedonian white-brined cheese, designated as Variants A, B, C, and D. Cheese samples were sourced directly from four different dairy processing facilities located in various regions of North Macedonia (Figure 1), ensuring diversity in production techniques.

To maintain the integrity of the samples, they were transported under temperature-controlled conditions on 5°C to the Certified Laboratory for Milk and Dairy Product Quality (LB Lact) in Plovdiv, R. Bulgaria. This laboratory is certified under international standards, ensuring the reliability and accuracy of all testing procedures.

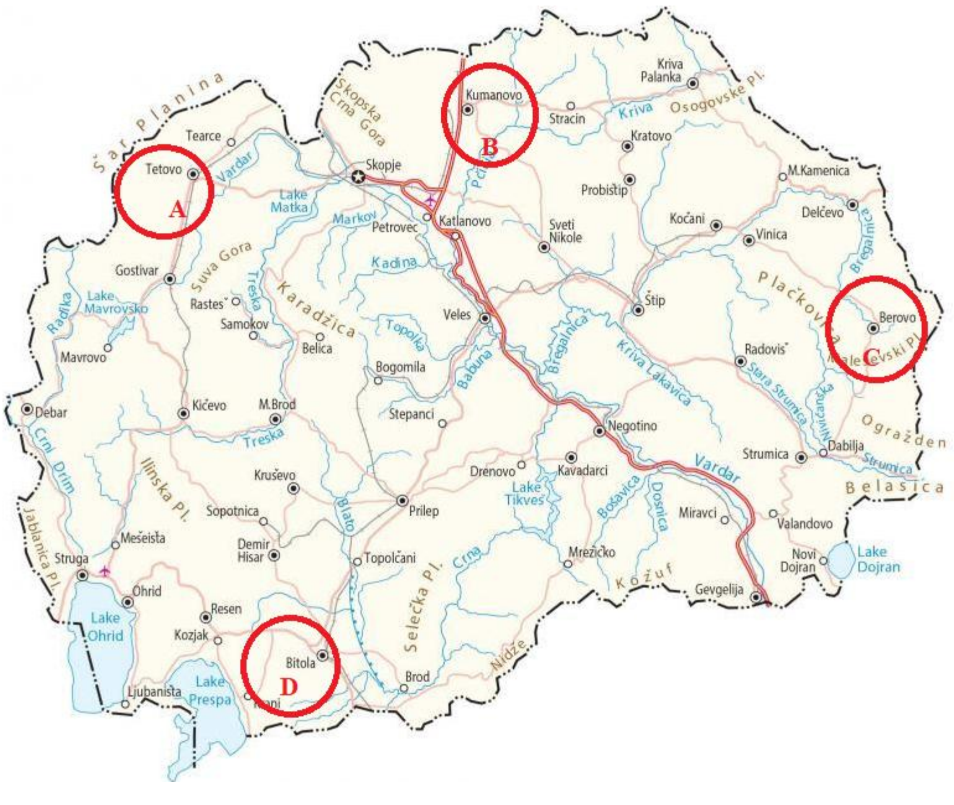

Figure 1: Map of researched area

Milk Fat Content Analysis. The milk fat content (%) in the cheese samples (Figure 2) was measured on the 8th, 20th, 30th, 40th, and 60th days of ripening according to ISO 3433:2008.

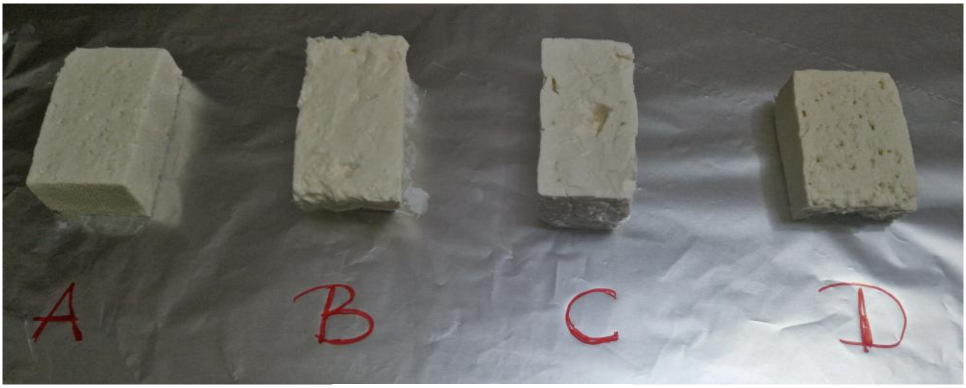

Figure 2: Cheese samples

RESULTS AND DISCUSSION

The comparative analysis made between the examined cheese samples for milk fat value is shown in table 1.

Table 1: Dynamics of Milk fat value in examined cheese samples

Milk fat value (%)				
Period of examination	Cheese Variant A	Cheese Variant B	Cheese Variant C	Cheese Variant D
Day 8	$23.88{\pm}0.07^{a}$	$23.80{\pm}0.10^{a,b}$	$22.67{\pm}0.05^{c}$	$24.60{\pm}0.05^{d}$
Day 20	$24.10{\pm}0.10^{a}$	$23.90{\pm}0.10^{b}$	$22.88{\pm}0.05^{c}$	$24.80{\pm}0.10^{d}$
Day 30	$24.36{\pm}0.15^{a}$	$24.0{\pm}0.10^{b}$	$23.0{\pm}0.10^{c}$	$25.07{\pm}0.05^{d}$
Day 40	$24.57{\pm}0.05^{a}$	$24.13{\pm}0.05^{b}$	$23.03{\pm}0.05^{c}$	$25.19{\pm}0.01^{d}$
Day 60	$24.63{\pm}0.20^{a}$	$24.18{\pm}0.03^{b}$	$23.11{\pm}0.02^{c}$	$25.41{\pm}0.02^{d}$

**Differences of values with different superscripts in the same row are statistically significant at level $p<0.05$;*

During the ripening process, significant differences $p<0.05$ in milk fat content were observed among the four cheese variants. On the 8th day, Cheese Variant D had the highest milk fat percentage (24.60±0.05%), while Cheese Variant C had the lowest value (22.67±0.05%). After 20 days of ripening, the milk fat content ranged between 22.88±0.05% for Variant C and 24.8±0.10% for Variant D. As ripening progressed, on the 30th day, a noticeable increase in fat content was recorded across all variants, with values ranging from 23.0±0.10% to 25.07±0.05%. This upward trend continued on the 40th day, with milk fat percentages between 23.03±0.05% and 25.19±0.01%.

By the 60th day, the milk fat content had stabilized, showing values between 23.11±0.02% and 25.41±0.02%, with Variant D consistently maintaining the highest milk fat level. Notably, Variant D had 0.78%, 1.23%, and 2.3% higher fat content than Variants A, B, and C, respectively. These findings were statistically significant ($p<0.05$) for most of the tested periods, suggesting that differences in technological processes and production conditions played a critical role in milk fat retention.

The higher milk fat content in Variant D can be attributed to variations in the raw milk composition, particularly the lack of milk fat standardization in Variants A, B, and D. In contrast, Variant C was produced using standardized milk fat (3.2%), which likely explains the greater deviations in fat content observed between the variants during ripening.

The gradual increase in milk fat content across all variants throughout the ripening period is consistent with findings from other studies. For example, Ivanov *et al.* (2016) reported an average fat content of 24.5±0.3% in Bulgarian white cheese after 45 days of ripening. Similarly, Chomakov *et al.* (2000) documented fat values ranging from 21% to 25% in white brined cheese produced from cow's milk. Further, Bojanić-Rašović *et al.* (2010) found an average fat content of 23.86% in Montenegrin white brined cheese, influenced by factors such as breed, feeding practices, and environmental conditions. Our findings align closely with those of Naydenova *et al.* (2013), who reported milk fat values between 22% and 23.25%, and Popović Vranješ *et al.* (2011), who identified fat content between 21.97% and 23.97% in Sjenica cheese. Additionally, Velevski (2015) observed milk fat content ranging from 22.34% to 22.40% in three varieties of white brined cheese, further supporting the results obtained in this study. Ismaili (2022) reported milk fat content in Macedonian white-brined cheese ranging from 23.20±0.01% to 25.03±0.03%, which aligns closely with the findings of this study. These values correspond to the range of milk fat percentages observed in the examined cheese variants during the ripening process, supporting the validity of the results obtained. Slightly lower milk fat values in white brined cheese were reported by Milenković (2017), who determined a milk fat content of 20.49±0.77% in cheese produced using industrial techniques. Similarly, Aydemir (2018) found an average milk fat content of 18.44±0.79% in "Beyaz" white brined cheese, while Beev *et al.* (2019) identified an average milk fat content of 16.4±3.071% in Bulgarian white brined cheese. These differences are most likely attributed to variations in the production techniques, conditions and the types of raw materials used in the manufacturing processes.

In this study, a novel functional model was developed to estimate milk fat values over specific time intervals during the ripening process, from the 8th to the 60th day. This model, represented by the equation $y=(AX^2+B)+(CX^2/D*X)$, is designed to apply to all examined variants, providing accurate predictions of milk fat content throughout the fermentation period.

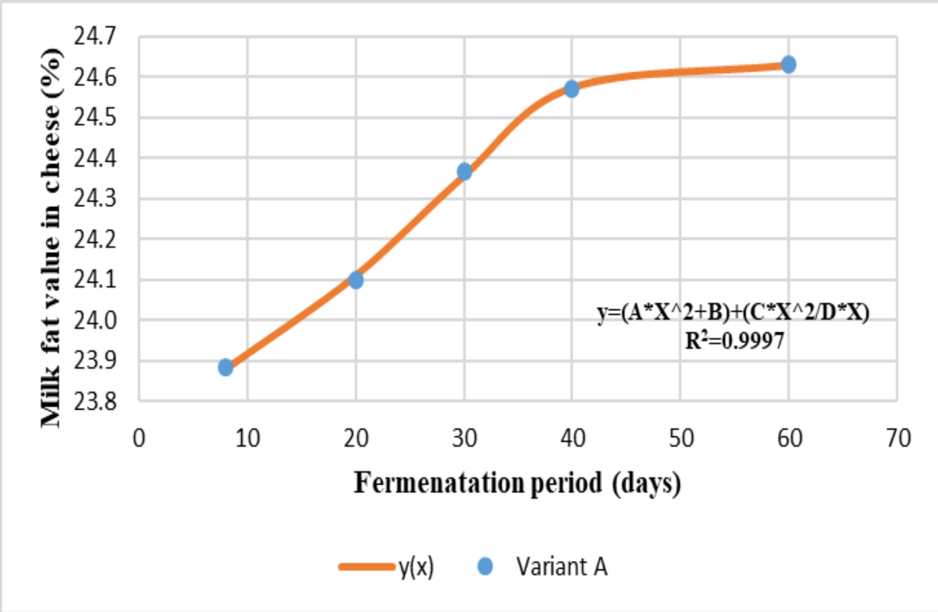

Figure 3: Milk fat value dynamics in cheese Variant A

For Cheese Variant A, the model parameters were determined as A=0.000958, B=23.8245, C=-0.00022, and D=18,322, yielding a highly accurate prediction with an R^2 value of 0.9997, indicating near-perfect correlation between the model's predictions and the experimental data. As shown in Figure 3, the dynamics of milk fat values in Cheese Variant A are illustrated with blue data points representing the experimental measurements, while the orange line depicts the predicted values generated by the functional model.

The model's high R^2 value underscores its potential as a valuable tool for accurately predicting milk fat levels under various production conditions, enhancing the ability to optimize cheese production processes based on technological and environmental factors.

The functional model developed for Cheese Variant B follows the same form, $y=(AX^2+B)+(CX^2/D*X)$, with specific parameters set as A=0.000423, B=23.77, C=-0.000035, and D=6.849. This model produced an R^2 value of 0.9984, confirming its high degree of accuracy and statistical significance in predicting the milk fat dynamics during the ripening process. The close fit between the model's predictions and the experimental data underscores its robustness and reliability. In Figure 4, the milk fat dynamics for Cheese Variant B are illustrated using green data points representing the experimental values, while the blue line indicates the model's predicted trend. The alignment between the experimental results and the model's curve demonstrates the effectiveness of this predictive tool in approximating the milk fat content during fermentation. The high R^2 value further validates the model, showing its capacity to account for the key variables influencing fat retention and distribution during cheese ripening.

This model is a valuable asset for optimizing cheese production processes and ensuring consistency in product quality.

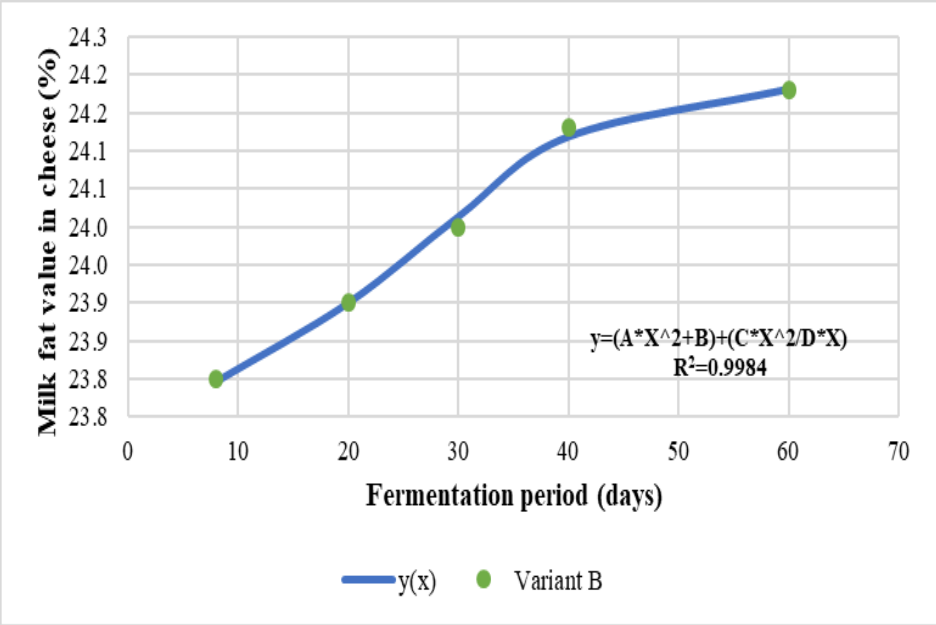

Figure 4: Milk fat value dynamics in cheese Variant B

The newly formulated model is also applicable to Cheese Variant C, represented by the equation $y=(AX^2+B)+(CX^2/D*X)$, with the following parameters: A=0.00051, B=22.68, C=-0.000001, and D=0.08. This model yielded an R^2 value of 0.9673, which confirms its statistical significance and reliability in predicting the dynamics of milk fat content during the fermentation process. In Figure 5, the dynamics of milk fat values for Cheese Variant C are depicted using orange data points that represent experimental measurements.

The blue line illustrates the Y(x) function derived from the predictive model, effectively approximating the parameters for milk fat during the fermentation period. The R^2 value indicates that while the model effectively captures the trends in milk fat content, it also suggests that additional factors may influence the variability in fat retention. This insight highlights the potential for further refinement of the model by incorporating additional variables related to production techniques and environmental conditions.

The same functional model is applicable for Cheese Variant D, characterized by the parameters A=0.00077, B=24.567, C=-0.000139, and D=15.39. This model achieved an impressive R^2 value of 0.9949, confirming its statistical significance and reliability in predicting the dynamics of milk fat content during the fermentation process.

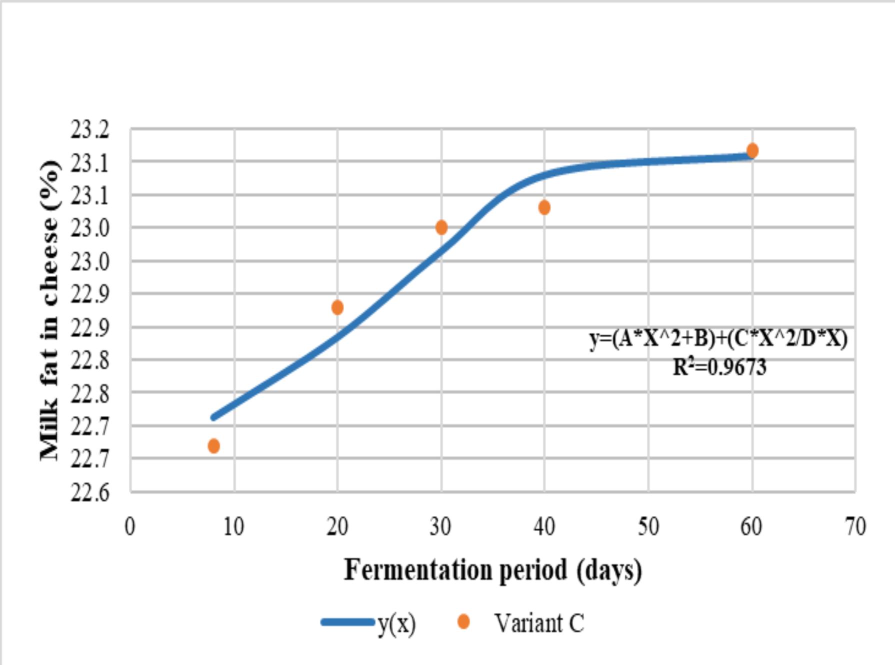

Figure 5: Milk fat value dynamics in cheese Variant C

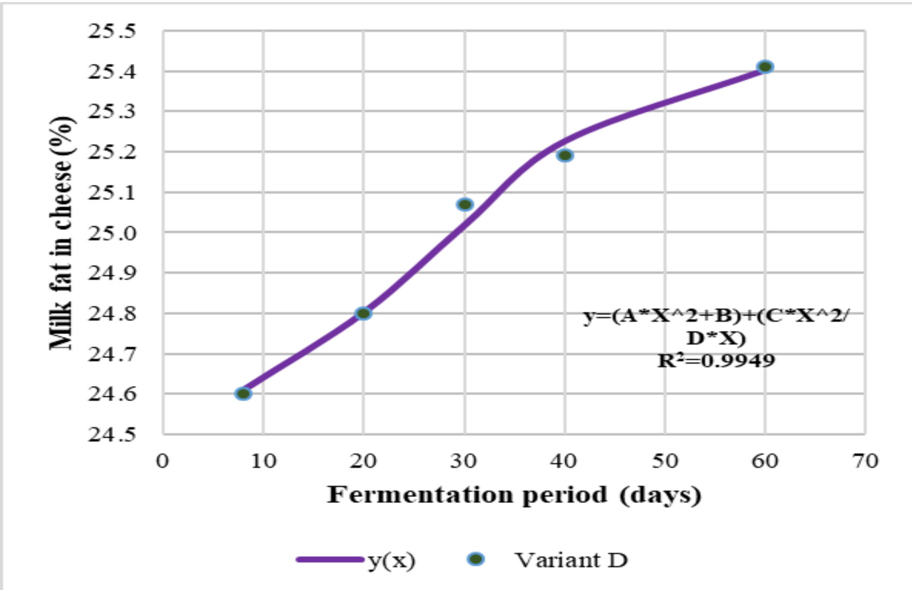

Figure 6. Milk fat value dynamics in Cheese Variant D

In Figure 6, the dynamics of milk fat values for Cheese Variant D are illustrated with green data points obtained from experimental measurements. The lavender line represents the Y(x) function derived from the predictive model, effectively approximating the parameters associated with the investigated phenomenon. The high R^2 value indicates that the model accurately captures the trends in milk fat content, demonstrating its efficacy in reflecting the underlying processes during cheese ripening. This model serves as a valuable tool for optimizing production practices and ensuring consistent quality in cheese products, potentially benefiting producers by facilitating real-time adjustments based on predictive insights.

CONCLUSIONS

The development of a predictive functional model for estimating milk fat values in Macedonian white-brined cheese provides significant insights into the complex processes of cheese ripening. The findings reveal that Cheese Variant D consistently exhibited the highest milk fat content, achieving a remarkable R^2 value of 0.9949. This suggests that specific technological and production conditions significantly impact fat retention during fermentation. Similarly, the strong predictive capabilities of the models for Variants A, B, and C highlight the robustness of this approach, with R^2 values ranging from 0.9673 to 0.9997 across the variants.

The observed differences in milk fat content among the variants, influenced by factors such as initial milk composition and production methods, underscore the necessity for careful management of these parameters to enhance product quality. The models not only provide accurate estimations but also offer a strategic tool for dairy producers aiming to optimize cheese production processes. By incorporating these predictive insights, producers can make informed decisions to enhance the sensory attributes and nutritional profile of their cheese products. In light of the growing demand for high-quality dairy products, these findings align with previous research indicating the critical role of milk fat in determining cheese quality and consumer acceptance. Ultimately, the successful application of this model could facilitate better resource management, improved quality control, and innovation in cheese production, thereby contributing to the sustainability and economic viability of the dairy industry.

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SUSTAINABLE BUCKWHEAT GROWING: AGROTECHNICAL AND ECONOMIC ASSESSMENT OF THE MINERAL FERTILIZERS' ROLE

SUMMARY

The sustainable cultivation of agricultural crops has become increasingly important, with innovations focused on increasing yields without harming the environment. In Ukraine, the use of biofertilizers and biopesticides is gaining popularity, though results vary due to specific application conditions. Organic fertilizers have shown clear benefits for many crops, but manure is not recommended for buckwheat. However, fertilizers containing effective microorganisms are promising, though they can be affected by adverse abiotic factors. This study aims to evaluate the impact of mineral fertilizers on buckwheat yield and to assess the economic viability of their use. Field experiments were conducted from 2021 to 2023 at Sumy National Agrarian University educational and research center, involving two buckwheat varieties, Slobozhanka and Yaroslavna, under different fertilization regimes. The study analyzed crop yield and economic efficiency under varying doses of NPK fertilizers ($N_{22}P_{22}K_{22}$, $N_{45}P_{45}K_{45}$, $N_{30}P_{45}K_{45}$ + N_{15}, $N_{50}P_{30}K_{70}$), as well as control (without fertilization). Results indicate that fertilizer use significantly improved yield compared to control, with the Slobozhanka variety showing higher responsiveness. The best economic outcomes were associated with moderate fertilizer use, balancing yield increase and input costs. Economic analysis shows that, while higher fertilization increased yields, it did not always result in higher profitability due to increased input costs, particularly for $N_{45}P_{45}K_{45}$. Excessive fertilizer use can also lead to long–term soil degradation, making balanced

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application crucial for sustainable production. Therefore, a strategic approach to fertilizer use is needed to optimize both yield and soil health, ensuring long–term agricultural sustainability.

Keywords: sustainability, yield, economic evaluation, soil health, fertilization strategies, profit.

INTRODUCTION

The issue of growing agricultural crops in a sustainable way is extremely relevant today (Datsko *et al.*, 2024; Mishchenko *et al.*, 2024; Trotsenko *et al.*, 2023). Under the term of sustainability, scientists mostly understand the ability to meet present needs without compromising the ability of future generations to meet their own needs. Increasingly, innovative approaches in agriculture are focused on boosting crop yields through means that do not harm the environment (Kovalenko *et al.*, 2024a; Kolisnyk *et al.*, 2024; Voitovyk *et al.*, 2024a). So, this could be called sustainable way of crop production. In Ukraine, the use of biofertilizers and biopesticides for pest and disease control is becoming more common (Radchenko *et al.*, 2024). However, the use of such products does not always lead to the desired effect due to specific conditions that must be met during their application. When it comes to organic fertilizers, the effect is usually clear and effective (Bhunia *et al.*, 2021; Tykhonova *et al.*, 2021). However, manure application for buckwheat is not recommended (Tao *et al.*, 2023; Radchenko *et al.*, 2023). At the same time, the use of fertilizers containing effective microorganisms is appropriate and effective. Yet, this type of fertilization does not always yield the desired results due to unfavorable abiotic factors that negatively affect microorganisms (Witkowicz *et al.*, 2020; Voitovyk *et al.*, 2024b). That is why a lot of agricultural producers are still using mineral fertilizers.

The economic evaluation of a technology or its individual components is a crucial criterion that helps farmers determine the impact of a particular practice on the crops they grow (Farooq *et al.*, 2016; Silver *et al.*, 2021; Kovalzhy *et al.*, 2024). The rising costs of fuel and agricultural production inputs have a significant impact on the industry’s economy (Dhakal *et al.*, 2015; Kovalenko *et al.*, 2024b). It is also important to understand the feasibility of their use and to consider the effect of a particular practice on economic efficiency and the environment. With limited budgets and significant environmental impacts, farmers must efficiently grow crops, especially buckwheat.

Government officials strive to support agricultural producers (Hryhoriv *et al.*, 2021; Vykliuk *et al.*, 2022; Williams *et al.*, 2022), but given the rising costs of fertilizers and fuel, farmers need to be particularly cautious when choosing optimal growing technologies. They should carefully assess the economic efficiency of each method to find the best balance between cultivation profitability, high yield, and environmental impact. Agricultural producers are encouraged to actively seek and implement innovative cultivation methods to reduce their dependence on fertilizer costs due to changes in the economic environment (Masoero *et al.*, 2021; Hryhoriv *et al.*, 2024). Moreover, when choosing fertilization and soil management methods, it is crucial to consider the ecological factor.

Therefore, the aim of this study was to examine the impact of mineral fertilization on buckwheat yield as well as to conduct an economic and environmental assessment of fertilizer use feasibility.

MATERIAL AND METHODS

Field studies were conducted at the Sumy National Agrarian University educational and research center from 2021 to 2023. A two–factor experiment was established, which included the following factors: varieties (Slobozhanka, Yaroslavna) and fertilization levels. The buckwheat (*Fagopyrum esculentum* Moench) cultivation technology involved several key stages, including plowing to a depth of 25–27 cm, harrowing to level the soil surface, and pre–sowing cultivation.

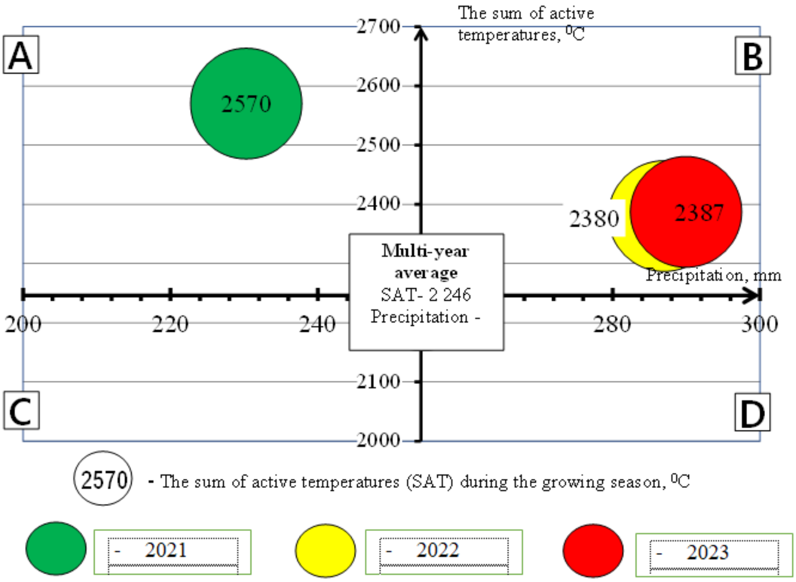

Figure 1. The sum of active temperatures (>10 °C) and the amount of precipitation during buckwheat growing season (May–August) compared to the average long–term values (meteorological station in the village of Sad Sumy region, Sumy district. GPS coordinates: 50.88095 Northern latitude, 34.71525 East longitude, 2021–2023). Conditions: A – warmer and drier; B – warmer and more humid; C – cool and dry; D – cool and wet.

Fertilization was carried out according to several schemes, including a control option without fertilizers and various fertilizer doses: $N_{22}P_{22}K_{22}$, $N_{45}P_{45}K_{45}$, $N_{30}P_{45}K_{45}$ + N_{15} and $N_{50}P_{30}K_{70}$. Harvesting was performed after most of the seeds had fully matured to prevent losses due to shattering. After harvesting, yield, grain quality, and the economic efficiency of the technology were evaluated.

The weather conditions during the vegetation periods of buckwheat plants (May–August) in the 2021–2023 research years showed significant differences compared to both the long–term average values and between each other (Figure 1).

To determine the economic efficiency of intensive cultivation of two buckwheat varieties, both value and natural indicators were used. Descriptive statistics were made at Microsoft Excel. The least significant difference (LSD) for buckwheat yield between varieties and fertilization treatments was calculated using the standard statistical approach based on the ANOVA model. LSD was determined at the 0.05 probability level to evaluate significant differences in yield across experimental treatments.

RESULTS AND DISCUSSION

Research results from 2021–2023 showed that buckwheat yield significantly depends on the level of fertilization and variety (Table 1). The Yaroslavna variety demonstrated an average yield of 1.47 t ha^{-1} without fertilizers, while increasing doses of mineral fertilizers led to higher yields. Specifically, with the recommended dose of $N_{45}P_{45}K_{45}$, the yield reached 1.78 t ha^{-1}, and with additional nitrogen feeding, the yield increased to 1.96 t ha^{-1}. However, at the calculated dose of $N_{50}P_{30}K_{70}$, the yield slightly decreased to 1.83 t ha^{-1}.

Table 1. Yield of different buckwheat morphotypes depending on fertilization level, 2021–2023

Varieties	Fertilization	Grain yield, t ha^{-1}			
		2021	2022	2023	Average
Yaroslavna	Control	2.17	1.30	0.95	1.47
	$N_{22}P_{22}K_{22}$	2.64	1.40	0.97	1.67
	$N_{45}P_{45}K_{45}$ – recommended	2.87	1.48	0.98	1.78
	$N_{30}P_{45}K_{45}$ + N_{15}	2.91	1.88	1.09	1.96
	$N_{50}P_{30}K_{70}$ calculated	2.93	1.54	1.02	1.83
Slobozhanka	Control	2.68	1.25	0.86	1.60
	$N_{22}P_{22}K_{22}$	2.68	1.41	0.88	1.66
	$N_{45}P_{45}K_{45}$ – recommended	3.21	1.46	0.89	1.85
	$N_{30}P_{45}K_{45}$ + N_{15}	3.29	1.62	0.92	1.94
	$N_{50}P_{30}K_{70}$ calculated	3.52	1.91	0.90	2.11
LSD_{05} for varieties, t ha^{-1}		0.33	0.36	0.05	0.24
LSD_{05} for fertilizes, t ha^{-1}		0.19	0.20	0.03	0.14

Compared to Yaroslavna, the Slobozhanka variety showed a higher average yield. Without fertilizers, this variety produced a yield of 1.60 t ha^{-1}, and with the use of both recommended and calculated doses of fertilizers, the figures significantly increased. The maximum yield was achieved with the calculated dose of $N_{50}P_{30}K_{70}$, reaching 2.11 t ha^{-1}, indicating a more effective response of this variety to fertilization.

The statistical analysis demonstrates the effectiveness of different fertilization treatments and buckwheat varieties on grain yield over three years (2021–2023). The inclusion of LSD values at the 0.05 probability level provides a reliable means to discern statistically significant differences between treatments and varieties. Specifically, the LSD for varieties (0.33 t ha^{-1}) and fertilization treatments (0.19–0.22 t ha^{-1}) highlights the sensitivity of the analysis in identifying meaningful yield variations, ensuring the robustness and validity of the experimental conclusions.

The total costs for cultivating the varieties using row planting without fertilizers amounted to € 258.62 ha^{-1} for the Yaroslavna variety and € 170.22 ha^{-1} for the Slobozhanka variety. These figures also included additional costs associated with the use of fertilizers. As a result, the cost differences between the options became even more pronounced.

Table 2 provides important data on the costs and revenues from cultivating the Yaroslavna variety depending on different fertilization options. Total costs increase with the use of various fertilizer regimes. The highest costs are observed in the options with the $N_{22}P_{22}K_{22}$ and $N_{45}P_{45}K_{45}$ fertilizer regimes, due to the high cost of the fertilizers.

Yield depends on the quantity and type of fertilizers applied, which affect both the quality and quantity of the harvest. Based on this, revenue from grain sales was calculated.

Table 2. Costs for cultivating the Yaroslavna variety using row planting depending on the fertilization regime

Indicator	Units of measurement	Control	$N_{22}P_{22}K_{22}$	$N_{45}P_{45}K_{45}$	$N_{30}P_{45}K_{45}$ +N_{15}	$N_{50}P_{30}K_{70}$
Cumulative costs	€ ha^{-1}	252.3	457.0	669.0	547.9	588.7
Nitroamofoska	€ ha^{-1}		204.6	416.7		
Ammonium nitrate	€ ha^{-1}				30.6	
Urea	€ ha^{-1}				34.3	56.6
Superphosphate	€ ha^{-1}				88.5	59.3
Potassium sulfate	€ ha^{-1}				142.1	220.5
Price of grain at sale	€ ha^{-1}	292.7				
Income	€ ha^{-1}	430.2	488.8	521.0	573.7	535.6
Profit	€ ha^{-1}	177.9	31.8	–148.0	25.8	53.1
Profitability	%	41.4	6.5	–28.4	4.5	9.9

The table on the costs of cultivating the Yaroslavna variety using row planting under different fertilization regimes indicates various profit levels per hectare for each option. Profit is calculated as the difference between income (the value of the harvested grain multiplied by yield) and the total cultivation costs. The highest profit is € 177.9 € ha^{-1}, achieved in the control fertilization option. This suggests that fertilizer costs were the lowest in this variant, leading to higher profits, even considering lower yields or other factors. Excluding the control option, the highest yield was obtained in the $N_{50}P_{30}K_{70}$ option.

The lowest profit was recorded with the $N_{45}P_{45}K_{45}$ fertilization regime, where profit amounted to – € 148 € ha^{-1}. This indicates that the total costs of fertilizers and other production expenses exceeded the income from grain sales, resulting in a loss.

Analyzing the data from the table on the costs of cultivating the Slobozhanka variety (Table 3) using row planting under different fertilization regimes, the highest total costs were observed in the $N_{45}P_{45}K_{45}$ and $N_{30}P_{45}K_{45}$ + N_{15} regimes, indicating high fertilizer costs in these combinations.

Table 3. Costs for cultivating the Slobozhanka variety using row planting depending on the fertilization regime

Indicator	Units of measurement	Control	$N_{22}P_{22}K_{22}$	$N_{45}P_{45}K_{45}$	$N_{30}P_{45}K_{45}$ +N_{15}	$N_{50}P_{30}K_{70}$
Cumulative costs	€ ha^{-1}	166.1	370.7	582.8	461.6	502.5
Nitroamofoska	€ ha^{-1}		204.6	416.7		
Ammonium nitrate	€ ha^{-1}				30.6	
Urea	€ ha^{-1}				34.3	56.6
Superphosphate	€ ha^{-1}				88.5	59.3
Potassium sulfate	€ ha^{-1}				142.1	220.5
Price of grain at sale	€ ha^{-1}	292.7				
Income	€ ha^{-1}	468.3	485.9	541.5	567.8	617.6
Profit	€ ha^{-1}	302.2	115.1	–41.3	106.2	115.1
Profitability	%	64.5	23.7	–7.6	18.7	18.6

The highest income is achieved in the option with $N_{50}P_{30}K_{70}$ fertilization background (€ 617.5 € ha^{-1}), which is related to the higher yield obtained using this fertilizer rate. The highest profitability level for fertilization backgrounds, excluding the control option, is also observed in the $N_{22}P_{22}K_{22}$ variant, confirming its financial effectiveness.

Regarding fertilizer costs, it is worth noting that certain types of fertilizers, such as superphosphate and potassium sulfate, have a significant impact on total costs and may vary depending on their prices and the quantities applied.

A comprehensive analysis of the costs of growing buckwheat varieties (Yaroslava and Slobozhanka) using row sowing methods, depending on different fertilization backgrounds, shows that the efficiency of cultivation significantly depends on the proper selection and application of fertilizers. The most profitable options are characterized by an optimal balance between total costs and revenue from the harvest.

However, it should be noted that the natural fertility of the soil plays an important role in achieving high profitability. Soils with a high level of natural fertility can provide the highest yields and. Accordingly, the greatest income without significant fertilizer costs. However, with intensive soil use without replenishing nutrients, depletion is possible.

Therefore although natural fertility is an important factor in buckwheat cultivation, the use of minimal fertilizers is still necessary to preserve soil fertility in the long term. Proper fertilizer use helps conserve soil resources, improve crop resilience to stress, and ensure consistently high yields year after year.

An ecological–economic assessment of buckwheat cultivation from 2021 to 2023 showed the importance of optimal use of mineral fertilizers to increase yields. The Yaroslava variety, without fertilizers, provided an average yield of 1.47 t ha^{-1}, while with the recommended dose of $N_{45}P_{45}K_{45}$, the yield increased to 1.78 t ha^{-1}. Similar results were observed for the Slobozhanka variety, where yields increased from 1.60 t ha^{-1} without fertilizers to 2.11 t ha^{-1} with the calculated dose of $N_{50}P_{30}K_{70}$.

The economic evaluation indicates that the most profitable options were those with minimal or control levels of fertilization, where costs were lower. For the Yaroslava variety, the maximum profit (177.9 € ha^{-1}) was achieved in the control option, while other options with higher doses of fertilizers (e.g., $N_{45}P_{45}K_{45}$) showed a decline in profitability due to high costs. For the Slobozhanka variety, the highest income was achieved with $N_{50}P_{30}K_{70}$ fertilization (617.5 € ha^{-1}), demonstrating the greater efficiency of this variety under intensive fertilization.

Findings of this paper are supported by the work of Bonilla-Cedrez et al. (2021), who highlight that addressing the ‘ecological yield gap’ in agriculture, particularly in sub-Saharan Africa, requires not only technological advancements but also economic considerations. Their study emphasizes that while the ecological yield gap represents significant untapped potential, the economic yield gap—the difference between current yields and profit-maximizing yields—remains a critical limiting factor. They further suggest that complementary strategies, such as improving soil fertility, reducing fertilizer costs, and spatially optimizing fertilization practices, are necessary to enhance both productivity and profitability in agricultural systems. The findings of this study align with the review by Folina et al. (2021), which emphasizes the critical role of nitrogen management in promoting sustainability within diversified farming systems. Nitrogen over-application often leads to low nitrogen use efficiency (NUE), despite high crop yields. Folina et al. (2021) highlight the potential of nitrification and urease inhibitors in improving nitrogen uptake, storage, and yield components while reducing nitrogen losses in the form of NO_3^- leaching and NH_3 emissions. Their work underscores the importance of integrating such inhibitors into fertilization schedules to enhance nitrogen-related indices, such as NUE, Nitrogen Agronomic Efficiency (NAE), and Nitrogen Harvest Index (NHI). This approach not only supports sustainable nitrogen management but also contributes to the profitability of farming systems through improved nitrogen supply and crop performance. Meanwhile, findings of Khan et al. (2022), demonstrated that the combined application of synthetic phosphorus fertilizers and organic amendments can significantly improve crop productivity and profitability. Their research highlighted the superior performance of single super phosphate (SSP) combined with farmyard manure (FYM), which enhanced key yield parameters such as grains per spike, thousand-grain weight, biological yield, and grain yield. Under full irrigation, SSP + FYM provided the highest benefit–cost ratio (BCR), indicating its economic viability. Furthermore, the integration of organic supplements with inorganic phosphorus sources not only improved wheat yield under optimal irrigation but also provided a sustainable strategy for soil fertility management and resource use efficiency.

From an environmental perspective, excessive fertilizer use can lead to soil depletion and reduced fertility in the long term. Therefore, it is important to maintain a balance between yield and ecological sustainability by applying fertilizers moderately to preserve soil fertility and avoid its depletion. Efficient fertilizer use helps conserve natural resources, stabilize yields, and enhance crop resilience.

CONCLUSIONS

The findings of this study highlight the critical balance between fertilization levels. Yield, and economic efficiency in buckwheat cultivation. Both the Yaroslavna and Slobozhanka varieties responded positively to mineral fertilizers, with increased yields under optimal fertilization conditions. However, the study also revealed that the most economically viable cultivation methods did not necessarily involve the highest fertilizer doses. For the Yaroslavna variety, the most profitable option was the control (unfertilized) approach, where lower costs led to higher profits, despite slightly lower yields. In contrast, the Slobozhanka variety showed the highest income with intensive fertilization using the $N_{50}P_{30}K_{70}$ scheme, though profitability varied across different regimes. This study underscores the importance of optimizing fertilization to enhance both productivity and cost–effectiveness. Excessive fertilizer use, while boosting yields, may reduce profitability due to high input costs and can pose environmental risks by contributing to soil degradation over time. Therefore, an integrated approach that balances fertilizer use with environmental sustainability is essential.

Such practices will help maintain long–term soil fertility, ensure consistent yields, and promote sustainable buckwheat cultivation. Ultimately, the study demonstrates that effective fertilization strategies must consider both the economic and environmental impacts, guiding farmers toward practices that maximize both yield and profitability while preserving the natural resource base for future cultivation.

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ORGANIC AGRICULTURE IN SERBIA- SHARE OF BARLEY IN IT

SUMMARY

Barley (*Hordeum vulgare* L.) (*Poaceae* family) is the most important type of cereal grown in temperate climate zones worldwide. It was one of the first cultivated grains. The areas under organic plant production are constantly increasing. Organic crop production in Serbia was performed on the area of 23,527 ha. In Serbia, in 2021, compared to other organic crops, organic fruit was produced the most, on 5,615 ha. In organic cereal production, the dominant role belonged to wheat, which was grown on the area of 1,581 ha in 2021. Organic wheat was followed by rye (879 ha) and spelt wheat (492 ha). Barley, with the area of 399 ha, ranked fourth. Based on data from the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, the paper analyzed and presented graphically the ten-year barley production in Serbia. Base and chain indices were used. In the 2011-2021 period, areas cultivated with organic barley varied, but not to the extent that could have been expected. The smallest area was recorded in 2014 with only 23 ha, and the largest in 2018 with an area of 445 ha. According to the regional distribution of areas under organic barley production in Serbia in the observed period (2011-2021) the largest areas were located in the Vojvodina region (on average about 260 ha).

Keywords: organic production, cereals, barley, areas

INTRODUCTION

Organic farming stands for a sustainable system of food production that maximises the use of renewable resources produced on the farm itself, does not harm the health of plants, animals and humans and protects the environment

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(Veličković *et al*., 2016). The organic farming sector has been experiencing permanent growth both globally and in Serbia for many years (Popović *et al*., 2017; 2020; Golijan and Živanović, 2017; Golijan *et al*., 2017). The fact that the area under organic crop production has quadrupled in the last ten years also contributes to this (Golijan *et al*., 2021).

One of the most important ancient cereal crops is barley. Since it was first domesticated about 10,000 years ago in the Middle Eastern region known as the Fertile Crescent, barley has played a very important role in the development of agriculture (Civan *et al*., 2021). The ancestor of domestic barley (*Hordeum vulgare* L.) is wild barley (*Hordeum spontaneum*). Today, barley is the fourth most important annual cereal crop in the world after wheat, maize and rice in terms of production volume and harvested area (Gozukirmizi and Karlik, 2017; Lakić *et al*., 2019; 2022; Rakascan *et al*., 2021; Božović *et al*., 2020; 2022). Barley is mainly grown for animal feed and malt for brewing. There are winter and spring barley, hulled and unhulled, and two-row and six-row varieties. Barley is characterised by a wide genetic divergence that provides its production under various agroecological conditions and its utilisations for different purposes (Pržulj and Momčilović, 2003). Compared to other cereals, barley is remarkably capable to adapt and acclimatise, with the ability to be grown at higher altitudes and latitudes, even under desert conditions. Therefore, barley is a primary source of food particularly in the regions where extreme climate conditions prevail, such as in the countries bordering the Himalayas, then in Ethiopia, Marocco, etc. (Lalić *et al*., 2018). Barley grain contains starch (65-68%), proteins (10-17%), β-glucan (4-9%), fats (2-3%) and minerals (1.5-2.5%) (Wang *et al*., 2015). Although barley was initially used as human food, it later began to be increasingly used as livestock feed, as well as in the malting process in the brewing industry. Recently, approximately two-thirds of grown barley are used as livestock feed, one-third in brewing industry, and only about 2% are directly used as food (Lalić *et al*., 2003). According to the FAOSTAT Report (2020) the global use of barley grain was 62% feed, 24% processing, 7% seed, 6% food and 1% other non-food uses. The need for a healthier environment and numerous adverse effects caused by current conventional agriculture have led to alternative movements in the agricultural development, among which are ecological systems, such as organic agriculture (Kovačević *et al*., 2011). Organic production preservers and improves soil biodiversity (Terzic *et al*., 2018; Simić *et al*., 2023; Stupar *et al.*, 2023; Sekulić *et al*., 2023; Popović *et al.*, 2024). Its methods control and increase soil fertility, protect the environment and apply the highest standards of plant and animal health protection (Ugrenović and Filipović, 2012; Ugrenović *et al*., 2021). According to Oljača *et al.* (2009), the research on the organic farming of barley is of great significance in Serbia, particularly in highland areas.

The objective of this study is to analyze the changes in the areas under organic barley production in the Republic of Serbia during the period 2011–2021, with a particular focus on regional distribution, fluctuations in cultivated areas, and the significance of organic barley production within the overall organic cereal production. Additionally, the study aims to provide insights into the economic aspects of production, market trends, and challenges in crop certification, highlighting the potentials and limitations of organic barley production in Serbia.

MATERIAL AND METHODS

The available literature data dealing with the issues of organic agriculture and the data provided by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (http://www.minpolj.gov.rs/organska/) were analyzed and graphically presented by the application of the Microsoft Excel 2010 program in order to observe the changes in the areas under organically produced barley in the 2011-2021 period. The following methods were used in the study: desk research, content analysis, comparative analyses and analyses of base and chain indices, and descriptive statistics (mean, standard deviation, coefficient of variation). Base indices express the percentage change in the level of the phenomenon in a given period in relation to its level in a specified base period. The general formula for calculating base indices is as follows: $B_i = \frac{Y_i}{Y_B} \cdot 100(\%)$. Base indices are calculated by dividing the value of the indicator in a given year by its value from the base year. The area cultivated with organic barley in 2011 was used as the base value. In this way, the relative change of the areas in relation to the base value was obtained. Chain indices are relative numbers (in %) that indicate changes in the state of the phenomenon over successive periods of time. Chain indices are calculated by dividing the value of the indicator in the observed year by its value in the previous year: $L_i = \frac{Y_i}{Y_{i-1}} \cdot 100(\%)$. That way, the relative change in the areas cultivated with this field crop was obtained in comparison to the previous year.

RESULTS AND DISCUSSION

According to the 2023 data of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, there was a noticeable trend of growth of areas cultivated with organic crops in the analyzed 2011-2021 period. Nevertheless, their share of the total area of agricultural land is very small - less than one per cent. In the last third of the observation period it fluctuated between 0.6 and 0.7 per cent. Organic crop production was performed on the area of 23,527 ha in Serbia in 2021, which was almost four times more than in 2011 Fig. 1.

In the first half of the analyzed period (2011-2016), the largest areas cultivated with organic crops belonged to cereals. These areas increased over the period from 1,211 ha (2011) to 4,607 ha (2016) (Golijan Pantović *et al*., 2022). The greatest increase was recorded in 2012, when the areas doubled in comparison with in the previous year. The next increases occurred in 2014 (24% greater than in 2013) and in 2016 (50.4% greater than in 2015). However, a change occurred in the 2017-2021 period. In 2017, compared to 2016, areas cultivated with cereals decreased by 946 ha, hence areas on which organic fruit was produced exceeded the area under cereals and ranked first.

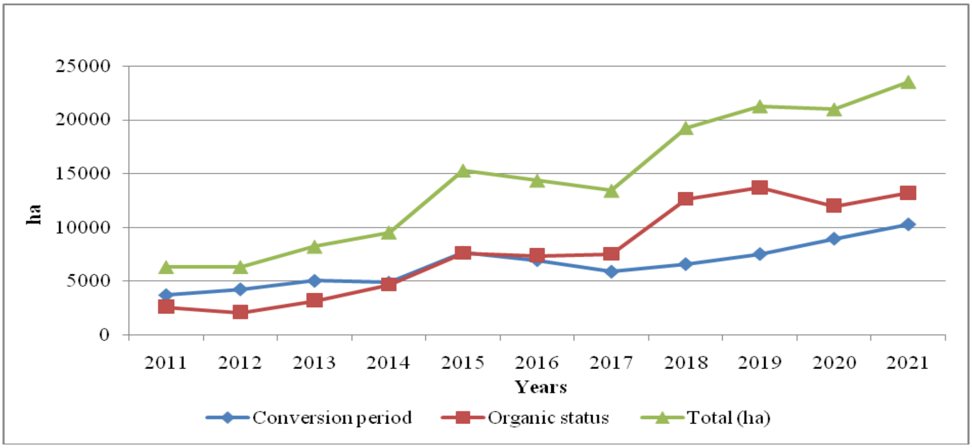

Figure 1. Land areas used for organic production in Serbia, 2011-2021

Observed over years, the areas cultivated with cereals decreased or increased, but they ranked second in the second half of the analyzed period, just behind orchards, except in 2020, when areas under fodder crops ranked second. It is expected that the global organic cereal market will grow at the rate of 9.2% in the period from 2018 to 2026 (Vlahović and Užar, 2021). Observed over regions in Serbia, cereal production was most represented in Vojvodina (Fig. 2).

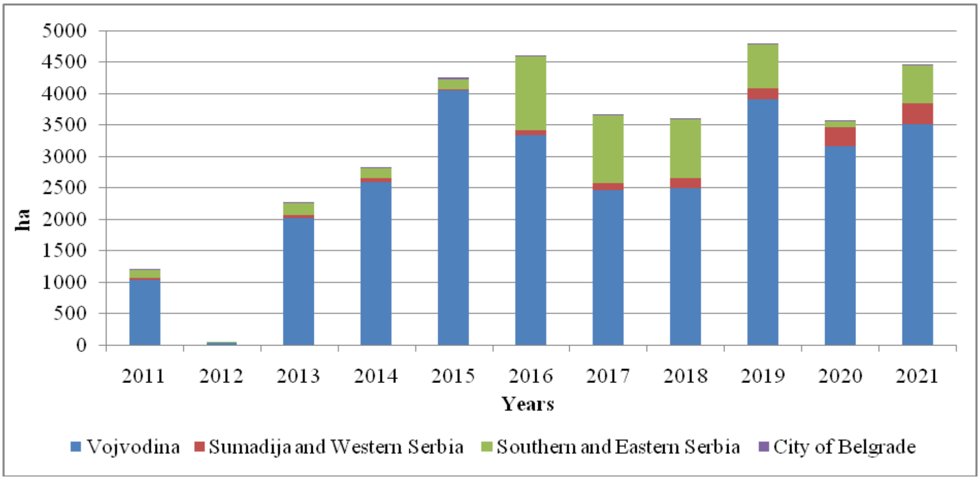

Figure 2. Land areas used for organic cereal production in the regions of Serbia, 2011 - 2021

Organic crop production in Serbia in 2021 was performed on the area of 23,527 ha, of which 17,003 ha were arable land, while 6,524 ha were under meadows/pastures. In 2021, the largest areas were under orchards (5,615 ha), then cereal crops (4,459 ha), fodder crops (3,054 ha), and industrial crops (2,122 ha).

Wheat, which is grown on the area of 1,581 ha, has a dominant role in organic cereal production. It is followed by rye (879 ha) and spelt wheat (492 ha). With the area of 399 ha in 2021, barley ranks fourth in organic cereal production (Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, 2023). Fig. 3 shows the trends in changes of barley in the total area under organic cereals during the observed period.

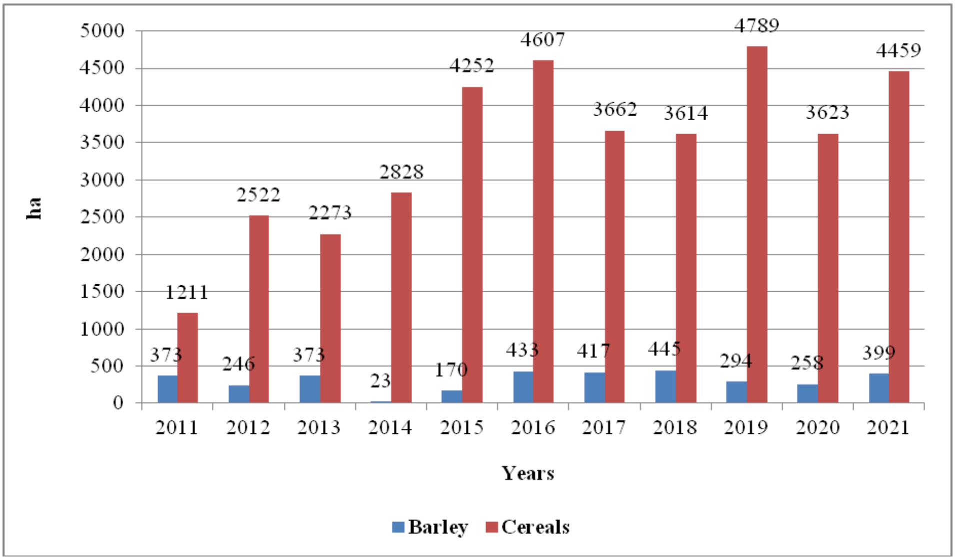

Figure 3. Share of land areas used for organic barley production in total land areas under organic cereals, 2011 - 2021

In the period from 2011 to 2021, areas under organic barley varied from 23 ha in 2014 to 445 ha n 2018. Their participation in the total areas cultivated with cereals also varied - it was highest at the beginning of the analyzed period in 2011, slightly more than 30%, when the areas under barley ranked second after silage maize. In 2014, the area cultivated with barley was the smallest. Back then, barley together with buckwheat, rye and millet, was among the least represented crops with the participation of only about 1%. The average share of areas under barley in the total areas under cereals amounted to about ten percents on average for the entire analyzed period. The changes in the areas under organic barley in Republic of Serbia during the analyzed period (2011-2021) is observable from the values of base and chain indices presented in Tab. 1.

The analysis of base indices shows that the largest reduction of areas in the relation to the base, initial year of 2011 was registered in 2014, when the areas were smaller by 93.9%. However, in the following year, 2015, this reduction was smaller by 54.5%. The highest increase was achieved in the following three years - 2016 (by 15.9%), 2017 (by 11.7%), and 2018 (by 19.3%). In the last year in the investigation period, 2021, the increase in comparison to the initial year of 2011, was of only 6.8%.

Table 1. Base and chain indices for land areas used for barley production, 2012 - 2021

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Areas (ha)	373	246	373	23	170	433	417	445	294	258	399
Base indices	100	66.0	99.9	6.1	45.5	115.9	111.7	119.3	78.8	69.2	106.8
Chain indices	100	66.0	151.3	6.1	748.3	254.9	96.3	106.8	66.0	87.9	154.3

The analysis of chain indices shows the alternating decreases and increases in the areas cultivated with organic barley. The largest decrease (by 93.9%) was recorded in 2014 compared to the previous year of 2013, while remaining decreases varied from 3.7% in 2017 in comparison to 2016, to 34% in 2012 in comparison to 2011, and in 2019 in comparison to 2018. On the other hand, the increases ranged from 6.8% in 2018 compared to 2017, to 648.3% in 2015 compared to 2014, which was also the largest increase in the analyzed period.

Based on the calculations using data from Table 1, it can be stated that the average area under organic barley in the Republic of Serbia, during the analyzed period from 2011 to 2021, was 311.8 hectares. The difference in areas under this cereal crop between the years with the largest areas (445.2 ha in 2018) and the smallest areas (22.7 ha in 2014) amounted to 422.6 hectares.The total areas under organic barley in the Republic of Serbia increased, on average, by 0.66% annually during the same period, while the average deviation from the average area under barley was 124.19 hectares. The variability of areas under barley in the Republic of Serbia was moderate and amounted to 39.83%.

During the entire observed period, the largest areas cultivated with organic barley were located in Vojvodina. Significant areas were cultivated with this crop in Southern and Eastern Serbia only in the 2016-2019 period. The largest areas (141 ha) were recorded in 2018, and the smallest ones in 2019 (66 ha) (Fig. 4).

In organic production, there are large variations in the production of all crops, because in order to obtain the organic standard, an application is made every year to the certification body to check the production method, i.e. whether the conditions for the standard are met (Golijan *et al.*, 2017). If something does not comply with the law on organic production, if the documentation is not complete, if residues of pesticides, mineral fertilizers, etc. are found, the certificate is lost and it is then considered conventional production. For example, it can therefore happen that the areas are 1000 ha in one year and 0 in another, because they are lost during the certification process (Golijan and Sečanski, 2021).

In the structure of the total export of organic products, by value, the participation of cereals is very limited. In 2020, only organically produced wheat was exported.

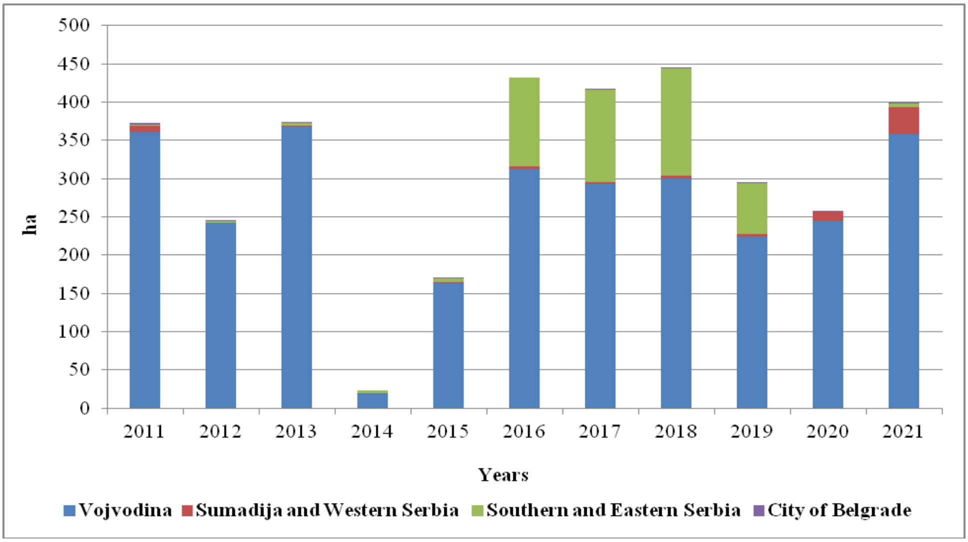

Figure 4. Land areas used for organic barley production in the regions of Serbia, 2011 - 2021

In EU countries, the category of agricultural products or their derivatives accounts for about 40% of imported organic products (1.1 million tonnes). Half of these are oil seeds, cakes and vegetable oils, and the largest category is cakes (mainly soybean meal). Another 0.3 million tonnes are rice, maize, wheat and flour (Willer *et al.*, 2022; www.organic-world.net).

In the paper of Miljatović *et al.* (2018), the costs and the results achieved in organic cultivation of wheat, barley and maize in Serbia were analyzed in order to determine the profitability of organic cultivation of these cereals. The market price of organic products is significantly higher than the price of conventional products (barley - 37.9 RSD/kg, wheat - 42.6 RSD/kg and maize - 45.0 RSD/kg). On the other hand, the yields achieved for wheat (5.8 t/ha) and barley (5.5 t/ha) are not far behind conventional production, while maize yields are significantly lower (4.8 t/ha). Income from subsidies is not a significant item of total income. Subsidies in conventional agricultural production amount to RSD 4,000/ha, while subsidies in organic agricultural production are 70% higher, amounting to RSD 6,800/ha. The maximum amount of government incentives is limited to RSD 136,000, i.e. 20 ha in organic farming. The highest profit per unit of capacity (1 ha) is achieved in wheat production (114,196.1 RSD/ha). Maize production comes second (94,726.6 RSD/ha), while barley production (59,450.9 RSD/ha) is the least successful according to this criterion.

It is a well-established opinion that organic farming may be one of the solutions to minimize negative externalities and to reduce agriculture's impacts on environment. Meier *et al.* (2015) and Tuomisto *et al.* (2012) documented that the yield in organic farming is usually lower than the one achieved with traditional cultivation.

CONCLUSIONS

The conducted study highlights the dynamic trends in organic barley production in the Republic of Serbia during the period 2011–2021. Despite fluctuations in cultivated areas, the overall trend indicates significant growth in organic crop production, reflecting the global rise in organic agriculture. Barley, as one of the most important ancient cereal crops, has proven its adaptability to diverse agroecological conditions, making it a valuable candidate for organic farming, particularly in Serbia. The analysis reveals that organic barley production experienced both substantial increases and occasional decreases in cultivated areas over the analyzed period. The largest areas were consistently observed in Vojvodina, emphasizing its role as the primary region for organic cereal production in Serbia. However, the share of barley within the total organic cereal production remained relatively modest, with an average of about 10% during the analyzed period. The largest decrease occurred in 2014, while the most significant growth was recorded in 2015. These variations reflect the challenges of maintaining certification and adhering to organic standards, which can significantly affect production levels year to year. Economic analysis confirms that organic barley production in Serbia faces challenges related to profitability and market trends. Compared to conventional farming, organic barley yields are competitive, but production costs and certification barriers remain significant obstacles. Nevertheless, the premium market prices of organic products and government subsidies for organic farming partially mitigate these challenges, although they are not substantial enough to fully offset the higher costs of organic production. Overall, organic barley production in Serbia demonstrates potential for growth but is constrained by structural and market-related challenges. To maximize its contribution to sustainable agriculture, further efforts should be directed toward improving certification processes, enhancing market access for organic products, and providing greater financial support to organic farmers. Organic farming, including barley cultivation, remains a promising pathway to reducing agriculture's environmental impact and promoting biodiversity while meeting the rising demand for organic products in both domestic and global markets.

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QUALITY OF VRANAC WINE OF THE RIJEKA SUBREGION IN MONTENEGRO - HARVEST 2019

SUMMARY

Montenegro has a very long tradition of growing vines and wine production. The production of very different wines with different characteristics is possible thanks to the very specific geographical location and the influence of the climate. In Montenegro, there are two autochthonous red grape varieties, Vranac and Kratošija. Vranac is the most important variety in Montenegro, but also in the region. The Rijeka subregion, which belongs to the Montenegrin basin region of Skadar Lake, has always been known as a very favorable area for the successful cultivation of vines and the production of quality wines.

This research covers Vranac wines from different producers in the Rijeka subregion produced in the 2019 harvest. The chemical composition (specific gravity, alcohol, extract, total and volatile acids, pH, total and free sulfur and reducing sugar) was examined. The phenolic composition (total polyphenols, anthocyanins, A_{280} index, color intensity and shade of wine, proportion of red, blue and yellow colors and the shape of the spectrum), as well as the sensory properties of the wine (color, smell, taste, etc.) was examed as well.

The obtained results showed a very good chemical and polyphenolic composition of the wines that were examined. According to the analysis results, the characteristics of each wine are a high content of alcohol and extracts, as well as anthocyanin and total polyphenols, whose values were also high. Sensory analysis showed that these are wines with good organoleptic properties and authenticity specific to this subregion.

Key words: Vranac wine, chemical and phenolic composition, sensory properties

INTRODUCTION

In Montenegro, the viticulture sector has been developing intensively in the last few years, so the number of registered producers, wineries, areas under

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vineyards and types of wine is increasing. When it comes to wine production and grape growing, red Montenegrin autochthonous varieties are dominant (among which the largest areas belong to Kratošija and Vranac). The production of wine and brandy is dominated by the Vranac variety (80%), followed by Kratošija and other international varieties (Pajović *et al*., 2014; Raičević *et al*., 2015; Pajović-Šćepanović *et al*.2016; Raičević *et al*., 2017; Pajović-Šćepanović *et al*., 2019, Raičević *et al*., 2020, Maraš *et al*, 2020, Popović *et al*., 2024). The wine-producing regions in Montenegro are: the Montenegrin basin of Lake Skadar (with the sub-regions: Podgorica, Crmnica, Bjelopavlići, Kuči, Katunski and Rijeka subregion), the Montenegrin-coastal region (which includes the Budva-Bar, Boka Kotor, Ulcinj subregions and the Adriatic hinterland), the region Nudo and the Montenegrin north (Dragaljsko polje, Šćepan polje, Bjelopolje and Nikšić subregions). The Rijeka subregion is located in the western part of the Montenegrin basin of Skadar Lake, located in the area between Skadar Lake and Lovćen. This subregion also includes the islands on Lake Skadar that the municipality of Cetinje includes. The altitudes at which wine producers are mainly concentrated are up to 100 m, and some at altitudes above 200 and 300 m (Study of the zoning viticultural geographic production area of Montenegro, 2017).

The Rijeka subregion has the potential to produce stronger wines with a moderately higher alcohol content, moderate acidity and a moderately higher to higher extract content. The research included localities within the Rijeka subregion: Dobrsko selo, Smokovci, Građani, Rvaši, Drušići, Meterizi and Ceklin.

The quality of wine, i.e. the physical-chemical and sensory characteristics of wine are formed under the influence of a large number of factors and in this respect each wine is different, so it is a great challenge for winegrowers and wine producers to maintain continuity in terms of quality and consistency of products (Ribéreau-Gayon *et al.* 2006; Roullier-Gall *et al.*, 2014; Mor *et al.*, 2022; Eder *et al.*, 2023). The choice of variety is a very important factor when moving into the production of wine, but the production itself depends on a number of external and internal factors that directly affect the final characteristics. It is for this reason that one variety, specifically Vranac, gives variations in the assessment of quality parameters by different producers, using different techniques of grape growing, processing and production (Ivanova et al., 2012; Pajović Šćepanović et al., 2019). Therefore, the condition for the best impact of terroir is that under the climatic conditions of the local area, a given variety ripens in a timely and complete manner (Banjanin, 2022).

The quality of wine mostly depends on the chemical composition of the must and the wine, not only in terms of the amount of individual ingredients, but also their relationship to each other (Ribereau-Gayon *et al.*, 2006; Raičević *et al.*, 2017). Phenolic compounds are extracted from grapes in the winemaking process. They have a positive effect on human health, among which are anticancer, cardioprotective, neuroprotective and antioxidant effects, and are responsible for the sensory characteristics of wine, especially color and taste (Raičević *et al.*, 2017; Pajović-Šćepanović *et al.*; 2019; Eder *et al.*, 2023).

All the listed factors, among which the most important are the variety, locality, i.e. the specificity of the wine region, the method of vinification and the aging of the wine determine the content of the chemical and polyphenolic composition, as well as the sensory properties of the wine. That is of great importance for characterizing and defining the ecological and oenological potential of the area for the production of quality wine (Pajović-Šćepanović *et al.*, 2024).

Rijeka region has good conditions for wine producing, but there is no data about wine quality. The aim of this research was to determine the quality parameters and define the phenolic and chemical composition, as well as the sensory characteristics of Vranac wines from different producers of the Rijeka subregion.

The Rijeka region offers favorable conditions for wine production; however, data on wine quality is lacking. This study aimed to evaluate the quality parameters and to characterize the phenolic and chemical composition, as well as the sensory attributes, of Vranac wines from various producers within the Rijeka subregion.

MATERIAL AND METHODS

In this paper, seven Vranac wines from different producers of the Rijeka subregion were examined, 2019 harvest. Rijeka subregion (Fig. 1 and Fig. 2) according to the Study of the zoning viticultural geographic production area of Montenegro, 2017. belongs to the Montenegrin basin region of Skadar Lake. Table 1. provides an overview of wines, producers and localities.

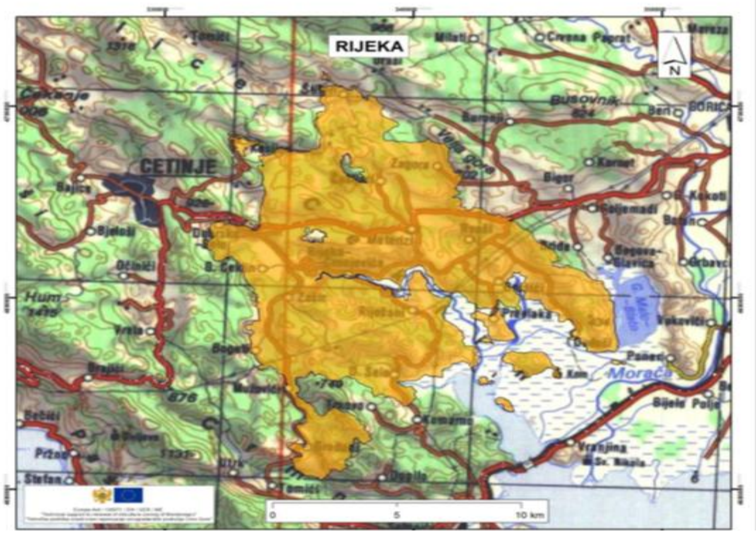

Figure 1. Map of the Rijeka subregion (Study of the zoning viticultural geographic production area of Montenegro, 2017)

Figure 2. Vineyards of the Rijeka subregion

Table 1. Tested wines Vranac of the Rijeka subregion, harvest 2019

Label Sample	Name of the wine	Wine producers	Locality
VZ	Vranac Zublja	Marković Winery	Dobrsko Selo
VS	Vranac Smokovac	Rakčević Winery	Smokovci
VT	Cormorant Tellus	Lipovac Winery	Građani
VM	Vranac Mrkan	Mrkan Winery	Rvaši
VJ	Vranac Janković	Janković Winery	Drušići
VE	Vranac Excellent	Vukmirović Winery	Meterizi
VV	Vranac Vukmirović	Vukmirović Winery	Ceklin

The Rijeka subregion is an area with exceptional climatic conditions for growing vines. The climate is modified Mediterranean, summers are hot and dry, winters rainy.The average annual temperature is 11°C. The average monthly precipitation of the Rijeka subregion is the lowest during June, 44.6 mm, and the highest is at the end of autumn and the beginning of winter, i.e. in December with an average value of 541.7 mm (MONSTAT, 2020). When it comes to air humidity, the average relative humidity is optimal, i.e. in the interval above 60% to 80%. The area of the Rijeka subregion is 14,394.64 ha.

Most of the wines included in this research,, were produced using the traditional method, which includes mulching grapes, spontaneous fermentation, draining the wine from the pomace and aging the wine. Among the wines produced by the classical process, VS wine differs in that in addition to selected yeast, enzyme and oak chips were added in fermentation. VZ wine is produced using vinifiers during alcoholic fermentation, under controlled conditions and aging in oak barrels. The VT sample is also characterized by a specific method of production compared to others from the Rijeka subregion, and that is the use of terracotta amphorae. After screening harvesting and mulching, the grapes in amphorae go through the maceration process for 30 days, and then they are also aged in amphorae for several months. One of the advantages of vinification in amphora is that it allows for so-called "soft" vinification.

1.Methods for determining physicochemical parameters

Chemical analyses that make up the experimental part of this work were performed in the laboratory of the Biotechnical Faculty in Podgorica. Physico-chemical analyses are determined by OIV methods (OIV Dec. 1990).

2.Spectrophotometric methods

Spectrophotometric measurements in our study were performed on the Varian Cari 100 spectrophotometer (Bio Tech, Maryland, USA).

3. Determination of the total phenolic content of wine

The total phenolic content in wine is determined spectrophotometrically, using the Folin-Ciocalteu method (Di Stefano and Guidoni, 1989*).* The method is based on the oxidation of phenolic compounds by the reagent Folin-Ciocalteu. This parameter is read at the wavelength: λ = 700 nm.

4.Determination of total anthocyanins

The content of total anthocyanins is determined by the spectrophotometric method of Di Stefano et al., 1989.The principle of the method is to measure the polymer pigments of wine after bleaching monomeric anthocyanins with bisulfite in an acidic medium (pH<1).The spectrum is determined in a 1 cm cuvette at wavelengths from 360 nm to 700 nm.

5.Determination of the A280 index

The amount of tannic substances (Index *A280*) is determined according to the Ribéreau-Gayon *et al*. method (1971). A sample of wine is diluted with distilled water and after that the absorbance is directly measured in the UV region of the spectrum.

6.Determining the color of wine

The color of wine consists of three components: yellow, red and blue (Puškaš, 2010). Yellow is read at a wavelength of λ=420 nm, red at λ=520 nm, and blue at λ=620. By measuring absorbance at these three wavelengths spectrophotometrically, six parameters can be calculated: wine color intensity and hue (Recueil d OIV, 1990), *the* proportion of yellow, red and blue, and the shape of the spectrum, according to the Glories method (1984) by directly reading the wine on a spectrophotometer.

7.Sensory analysis

The sensory analysis was conducted by a commission of 7 professional, licensed tasters, who completed the training and passed the exam of the evaluator's ability for the sensory evaluation of wine. The wines (30 ml) are presented in standard wine glasses (ISO 3591:1997, Sensory analysis – Apparatus - Wine-tasting glass). The sensory characteristics of the wines were assessed according to the 100-point system (OIV): a maximum of 15 points for appearance, a maximum of 30 points for aroma, a maximum of 44 points for taste and a maximum of 11 points for harmony (Resolution OIV/concours 332A/2009 OIV standard for international wine and spirituous beverages of vitivinicultural origin competitions. Paris, France: Inter-national Organisation of Vine and Wine, 2009). The results of the sensory evaluation were obtained as the mean of the points of all tasters.

8.Statistical processing of results

Statistical data processing was determined by the computer program STATISTICA (data analysis software system), version 10, using standard mathematical-statistical methods that include analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Chemical composition of the tested wines Vranac

The results of chemical analyses (specific gravity, alcohol, extract, total and volatile acids, free and total sulfur, pH value and reducing sugar) of Vranac wine samples for the 2019 harvest are shown in Table 2.

The Chemical composition of the wine showed variability between all the parameters of the tested samples. The alcohol content in the tested samples ranges from 12.7 % vol in VJ to 15.00 % vol in VS and VE samples. The average alcohol value is 13.8 % vol., which is in line with the results of Raičević *et al.*, (2014), where alcohol values of 12.8 to 13.9 vol% were recorded, and Pajović-Šćepanović *et al.* (2016) where values range from 12.9 to 13.7 % vol. all the wines had a high alcohol content.

The content of extracts in the wines was also high. The mean value for the total extract is 29.6 g/l, which corresponds to the extract content in the wines of the Rijeka subregion according to the Reionization Study (2017). The average value of total acids in the tested samples was 5.7 g/l, which is in line with the research of Raičević *et al.* (2014), where the value ranged from 5.5 to 5.98 g/l. and Pajović-Šćepanović *et al.*(2016) with values ranging from 4.7 to 5.8 g/l. The average value of volatile acids in the samples was 0.7 g/l, while the average pH value was 3.55. Free SO_2 ranged from 27.54 mg/l for VJ to 31.65 mg/l for VT, indicating that the wines were more sulphurized. The mean value of free SO_2 was 29.4 mg/l and total SO_2 was 115.18 mg/l all tested wines belong to the category of dry wines. Statistical analysis (ANOVA) showed that there was a statistically significant difference between all parameters of the chemical composition of the examined wines (p<0.05), which indicates the influence of different vinification methods.

Table 2. Chemical composition of tested wines, 2019 harvest

Sample	**Relative density (g/ml)**	**Akohol (%vol)**	**Total extract (g/l)**	**Total acids (g/l)**	**Volatile acids (g/l)**	**Ph**	**Free SO_2 (mg/l)**	**Total SO_2 (mg/l)**	**Reducing sugar (g/l)**
VZ	$0,9900^{a}$	$13,5^{a}$	$27,1^{a}$	$5,9^{a}$	$0,49^{a}$	$3,35^{a}$	$28,31^{a}$	$119,79^{a}$	$1,65^{af}$
VS	$0,9964^{b}$	$15,0^{b}$	$39,1^{b}$	$6,5^{b}$	$0,97^{b}$	$3,58^{bc}$	$30,00^{b}$	$138,73^{b}$	$4,0^{b}$
VT	$0,9910^{a}$	$13,9^{a}$	$28,7^{cf}$	$5,7^{ac}$	$0,75^{c}$	$3,60^{bc}$	$31,65^{c}$	$117,48^{c}$	$2,05^{ce}$
VM	$0,9970^{b}$	$13,00^{c}$	$35,1^{d}$	$5,3^{c}$	$0,70^{c}$	$3,63^{b}$	$30,09^{b}$	$106,34^{dg}$	$1,42^{d}$
VJ	$0,9910^{a}$	$12,7^{c}$	$20,0^{e}$	$5,7^{ac}$	$1,0^{b}$	$3,65^{b}$	$27,54^{a}$	$114,03^{e}$	$2,06^{e}$
VE	$0,9930^{ab}$	$15,0^{b}$	$29,2^{f}$	$5,8^{a}$	$0,95^{b}$	$3,50^{c}$	$27,95^{a}$	$108,91^{f}$	$1,63^{f}$
VV	$0,9900^{a}$	$13,5^{a}$	$27,0^{a}$	$5,3^{c}$	$0,52^{a}$	$3,57^{bc}$	$30,20^{bc}$	$105,06^{g}$	$2,50^{g}$
Mean	*0,9926*	*13,8*	*29,6*	*5,7*	*0,7*	*3,55*	*29,4*	*115, 8*	*2,18*

* different lowercase letters indicate statistically significantly different values of the analyzed parameters (p<0.05) of the tested wines (p < 0.05); * ns - no significance p>0.05; * A-F significance p<0.05;

Polyphenolic composition of the tested wines Vranac

The determinated parameters of the polyphenolic compositionare: total polyphenols, A_{280} index, total anthocyanins, and color intensity and shade. The values obtained by spectrophotometric measurements are shown in Table 3 and Table 4.

The highest content of polyphenols was recorded in VE wine (2144 mg/l), followed by VZ wine (1735 mg/l) and VM wine (1678 mg/l). With the lowest content of polyphenols was VV wine (934 mg/l), which was assessed as statistically very lower content compared to other tested wines. The average polyphenol content of the tested samples of Vranac wine is 1548 mg/l, is in line with the results of Raičević *et al.* (2017), where the values of total polyphenols in wines produced by the traditional method were measured from 1950 to 2250 mg/l and the results of Pajović Šćepanović *et al.*(2019), who cited range from 1832 to 2182 mg/l and Sošić *et al*, 2023 in the range of 1020 mg/l g/l to 2140 mg/l.

The highest value of anthocyanins was measured in VS wine (843 mg/l), while the lowest value was VT (414 mg/l). The higher content of total anthocyanins in VS wine can be explained by the use of enzymes and oak chips during fermentation, which probably caused a higher extraction of coloring substances from the solid parts of the grapes into the wine. The average value of the total anthocyanins of the tested wine samples is 530 mg/l. The results are in line with the research of Raičević *et al.* (2017), where total anthocyanins range from 600-870 g/l for the 2008 harvest year and 640-790 mg/l for 2009. A similar range of total anthocyanins was determined in the study by Pajović-Šćepanović *et al.* (2019) for 2016 (738 mg/l - 883 mg/l). The content of total anthocyanins in our study is higher than that of other studies, such as Košmerl *et al.* (2013) where values range from 201 to 237 mg/l and Mitrevska *et al.* (2020) with an interval of 121 to 273 mg/l, and lower than the average value obtained by Sošić *et al*. 2023 (805 mg/l). Statistical analysis (ANOVA) showed that there was a statistically significant difference between the values of total polyphenols, A_{280} index and total anthocyanins ($p<0.05$) in the tested wines.

Table 3. Content of total polyphenols, A_{280} index and total anthocyanins in wines, 2019 harvest

Sample	Total polyphenols (mg/l)	Index A_{280}	Total anthocyanins (mg/l)
VZ	1735^{a}	$78{,}09^{a}$	551^{a}
VS	1461^{b}	$80{,}38^{b}$	843^{b}
VT	1439^{c}	$64{,}14^{c}$	414^{c}
VM	1678^{d}	$66{,}63^{d}$	490^{d}
VJ	1447^{e}	$58{,}43^{e}$	424^{e}
VE	2144^{f}	$75{,}43^{f}$	527^{f}
VV	934^{g}	$42{,}59^{g}$	464^{g}
Mean	*1548*	*66.50*	*530*

* different lowercase letters indicate statistically significantly different values of the analyzed parameters ($p<0.05$) of the wine ($p < 0.05$); * ns - no significance $p>0.05$; * A-F significance $p<0.05$;

Table 4. Results of colorimetric parameters of wine, harvest 2019

Sample	Color Intensity (IB)	Color Shade (NB)	% of the red color	10% of the yellow color	10% of the blue color	Spectrum shape (dA%)
VZ	$1{,}65^{a}$	$0{,}72^{a}$	$50{,}01^{a}$	$35{,}80^{a}$	$14{,}18^{a}$	$65{,}95^{a}$
VS	$2{,}72^{b}$	$0{,}80^{ab}$	$45{,}02^{bc}$	$17{,}51^{b}$	$7{,}47^{b}$	$76{,}90^{b}$
VT	$1{,}20^{cd}$	$0{,}90^{b}$	$44{,}68^{c}$	$40{,}04^{c}$	$15{,}28^{c}$	$88{,}21^{c}$
VM	$1{,}44^{ad}$	$0{,}81^{ab}$	$47{,}39^{df}$	$38{,}26^{d}$	$14{,}35^{ad}$	$74{,}21^{d}$
VJ	$1{,}08^{c}$	$0{,}96^{b}$	$43{,}70^{e}$	$41{,}82^{e}$	$14{,}47^{d}$	$85{,}63^{e}$
VE	$1{,}50^{a}$	$0{,}81^{ab}$	$47{,}33^{f}$	$28{,}13^{f}$	$14{,}53^{d}$	$85{,}08^{f}$
VV	$1{,}09^{c}$	$0{,}71^{a}$	$48{,}47^{g}$	$35{,}02^{g}$	$16{,}60^{e}$	$85{,}26^{f}$
Mean	*1,52*	*0,81*	*45,65*	*35,22*	*13,83*	*80,17*

* different lowercase letters indicate statistically significantly different values of the analyzed parameters ($p<0.05$) of the wine ($p < 0.05$); * ns - no significance $p>0.05$; * A-F significance $p<0.05$;

The average value for color intensity is 1.52 (Table 4). The obtained values are lower than those obtained in other studies, such as in Košmerl *et al.* (2013) (5.33-6.01), Raičević *et al.* (2017) (4.25-6.29) and Pajović-Šćepanović *et al.* (2019) (2.24-4.45) and higher than the values presented in the research by Sošić *et al.* (2023), where the value was 1.41. The average color shade value is 0.81. The values of the shade of the color are higher than the values that are found in Košmerl *et al.* (2013) range from 0.21-0.26, Raičević *et al.* (2017) from 0.25 to 0.31, Pajović-Šćepanović *et al.* (2019) in the range of 0.27 to 0.46 and the average value stated by Sošić *et al.* (2023), which was 0.59. The proportion of dye (A420%, A520% and A620%) represents the percentage share of each of the components of the dye, while the value of dA (%) refers to the "brilliance" of the red color *(*Puškaš, 2010*)*.The mean values for the color content are: red - 46.65%; yellow - 35.22%; blue - 13.83%. The highest value of dA%, i.e. brightness was measured in VT (88.21%), and the lowest in VM (65.95%), while the average value is 80.17%. The statistical analysis confirms a significant difference between the values of the colorimetric parameters of the tested wines ($p<0.05$), especially in the proportion of yellow color and the shape of the spectrum (dA%).

Sensory characteristics of the tested wines Vranac

Sensory evaluation included the main components of the wine's qualities: appearance (clarity and color), aroma (purity, intensity and quality), taste (purity, intensity, persistence and quality) as well as harmony or general impression. The results of sensory assessment are shown in Table 5.

Based on the points from Table 5, it can be seen that the average overall sensory analysis scores of the tested wines ranged from 75.2 (VJ) to 85.6 (VS). The highest grade was given to the wine VS of the Rakčević winery due to its drinkability, fullness of taste and authenticity, which indicates that the proper use of oenological agents, with quality and well-processed grapes, is one of the most important conditions for the production of quality wine. Statistical analysis showed a significant difference in the overall sensory score between wines obtained by modern vinification methods compared to wines produced in the traditional way ($p<0.05$). The wines VZ, VS and VT received an overall score of over 85 points, which is a significantly higher score compared to the other wines examined and which places them in the category of premium wines.

As for the individual parameters for sensory analysis, the scores for the appearance of the wine were in the range of 11.4 to 13.0 and statistically did not differ significantly. The highest score was given to VS wine (13 points). The grades for the clarity of the wine ranged from 3.8 to 4.4, and for the color from 7.6 to 9. Sensory scores for nose ranged from 24 to 26.8. By alising the statistical points, a certain variability in the values is evident. The highest marks for cleanliness, smell and quality went to VS, the Rakčević winery. In this wine, we have a pronounced complexity in the form of an abundance of ripe fruity aromas of small berries, blackberries and blueberries, with discreet dry spices.

Table 5. Results of sensory evaluation of wine

Sample	Visual		Nose			Taste				Harmony	TOTAL
VZ	Limpidity	Aspect other than limpidity	Genuineness	Intensity	Quality	Genuineness	Intensity	Harmonious persistence	Quality		
	4,2	8,4	5	7	13,8	4,6	6,8	6,8	18,4	10	85,2
	12,8^{a}		**25,8^{ac}**			**36,6^{a}**				**10^{a}**	**85,2^{ab}**
VS	4	9	5,4	7	14,4	4,6	6,6	7	17,8		
	13,0^{a}		**26,8^{b}**			**36^{bd}**				**9,8^{a}**	**85,6^{a}**
VT	4	8,6	5,2	6,2	14	5	7	7	18,4		
	12,6^{a}		**25,4^{ae}**			**37,4^{c}**				**9,7^{a}**	**85,1^{b}**
VM	4,4	8,4	5,2	6,8	14	4,8	6,6	6,7	17,8		
	12,8^{a}		**26,0^{c}**			**35,9^{d}**				**9,7^{a}**	**84,4^{c}**
VJ	3,8	7,6	5,6	6,4	12	4,2	6,1	5,3	15,4		
	11,4^{b}		**24,0^{d}**			**31,0^{e}**				**8,8^{b}**	**75,2^{d}**
VE	4,4	8,4	5	6,8	13,2	4,6	6,6	6,2	17,2		
	12,8^{a}		**25,0^{e}**			**34,6^{f}**				**9,2^{b}**	**81,6^{e}**
VV	4,2	8,4	5,2	7	13,2	4,8	6,2	6,4	16		
	12,8^{a}		**25,4^{ae}**			**33,4**				**9,2^{b}**	**85,2^{ab}**
Mean	***12,5***		***25,5***			***35,0***				***9,5***	***82,5***

* different lowercase letters indicate statistically significantly different values of the analyzed parameters ($p<0.05$) of the wine ($p < 0.05$); * ns - no significance $p>0.05$; * A-F significance $p<0.05$;

When it comes to the taste of wine, which consists of four components (purity, intensity, persistence and quality), a statistically significant difference in points is evident in almost all wine samples. VT wine received the highest score for taste due to the specific process of maceration and aging in amphorae, which gives it specificity and recognizability, with a complex taste and an excellent balance of acids and tannins. The highest score for harmony (10), i.e. The general impression of VZ wine is probably the result of the method of production in vinifiers, where fermentation took place under controlled conditions. The wine is full-bodied, with persistent acids, delicate tannins and spicy notes, which are the result of aging in oak barrels.

CONCLUSION

Autochthonous varieties are of great importance for the promotion of Montenegrin viticulture and winemaking, and the most important red variety is Vranac - a variety of extremely good yield and great technological potential, which certainly positions it at the very top in terms of distribution, not only in Montenegro, but also beyond.

Based on the parameters of the chemical composition of the tested wines, we concludeed that all wines have excellent characteristics, typical for the climate of the Rijeka subregion, which are characterized by a high content of alcohol and extract. A higher content of total acids was also recorded, which is of great importance for microbiological stabilization, but also contributes to the freshness of the wine. All tested wines belong to the category of dry and are stable in terms of possible side effects in the phase of subsequent fermentation and aging of wine. Other tested parameters are within the expected limits.

The analyses revealed a high content of total polyphenols in the wines of the Vranac subregion of Rijeka (1548 mg/l) and a high content of total anthocyanins (530.13 mg/l), as well as other colorimetric parameters.

Sensory analysis has shown that these wines have mostly a very pronounced ruby red color, with fruity aromas, predominantly moderate to full-bodied. Most of the tested wines received an overall score of over 85 points, which puts them in the category of top wines.

Different methods of vinification (vinification in controlled conditions, maceration and aging in amphorae, traditional way with and without the use of oenological agents) have left a mark on the chemical and polyphenolic composition, as well as the sensory quality of the tested wines, but regardless of the differences, it is evident that these are very authentic and striking wines, with pronounced characteristics of the Vranac variety. It can be concluded that the Rijeka region offers favorable conditions for red wine production.

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DETERMINATION OF HEAVY METALS CONTENT IN *Achillea millefolium L.* AND RESPECTIVE INFUSIONS

SUMMARY

In this paper, the content of heavy metals (Cu, Mn, Fe, Co, Ni, Pb, Zn) was determined by Flame Atomic Absorption Spectrometry (FAAS) in the leaves of *Achillea millefolium L*., as well as in herbal tea infusions prepared out of them. Samples are taken from selected locations in Bosnia and Herzegovina, including five urban and five rural areas. The content of analyzed metals in *Achillea millefolium* leaves in samples from urban areas was in the range: <LOD (Ni); <LOD – 0,132 mg/kg (Co); 0,684 – 3,92 mg/kg (Pb); 4,47 – 8,79 mg/kg (Zn); 7,42 – 10,14 mg/kg (Cu); 46,16 – 100 mg/kg (Mn); 82,14 – 143 (Fe). The content of analyzed metals in the leaves of *Achillea millefolium* in samples from rural areas was in the range: <LOD (Ni); <LOD – 0,435 mg/kg (Co); 5,84 – 11,54 mg/kg (Cu); 7,43 – 24,76 mg/kg (Zn); 1,24 – 77,72 mg/kg (Pb); 62,54 – 203 mg/kg (Mn); 52,14 – 246 mg/kg (Fe). The content of analyzed metals in tea infusions prepared from *Achillea millefolium* from all urban and rural locations ranged from: <LOD (Co); 0,172 – 0,659 mg/kg (Pb); 0,467 – 1,81 mg/kg (Ni); 0,865 – 1,95 mg/kg (Cu); 1,58 – 4,75 mg/kg (Zn); 1,81 – 5,67 mg/kg (Fe); 7,90 – 34,68 mg/kg (Mn). By comparing the content of heavy metals in the leaves of *Achillea millefolium* collected from urban and rural locations, a wide variation of the content was observed, depending on the sampling locations and the potential natural or anthropogenic sources of metals nearby. The results of metal content in herbal tea infusions of the *Achillea millefolium* suggested that the metals are mostly present in water-insoluble forms.

Keywords: *Achillea millefolium*, heavy metals, water infusion, urban and rural locations, FAAS

INTRODUCTION

Plants represent a direct or indirect source of minerals in the human diet. The species that are utilized to produce a variety of phytopreparations used in the

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pharmaceutical industry-which are available as single- or multi-component teas and are extensively used in traditional medicine-are especially significant. Wildcrafted plants (more than 200 species) and cultivated plant (around 30 species) are sources of medicinal raw materials (Živkov-Baloš et al., 2014). Herbal teas are the primary application for medicinal herbs. The complex chemical composition of plant includes flavonoids, alkaloids, enzymes, minerals, and trace elements (Ražić and Kuntić, 2011). Kabata Pendias (2000), in a study mentioned that medicinal plant can accumulate more heavy metals such as Cd, As, Pb, and Hg compared to other plants. Mineral elements like Ca, Cu, Fe, K, S, and Zn, which are vital to human physiology and some of which are frequently lacking or present in traces in human diets, can be found in tea plants. However, while vital elements like Cu, Fe, and Zn might influence various biochemical pathways based on the intracellular concentration, the presence of As, Cd, Ni, and Pb can be concerning due to their significant toxicity, even at low levels (Fernandes et al., 2022). Heavy metals enter the environment from natural and anthropogenic sources. Medicinal plants growing in nature can accumulate heavy metals depending on their individual properties, metal concentration in soil, air, and water, climatic factors, and other environmental factors. Metal accumulation in plants is mainly governed by two processes, which involve the uptake of metals into plant cells and their translocation from the roots to other parts of the plant. In hyperaccumulating plants, the highest percentage of heavy metals in plants is transported from the roots to the stem, so the concentration of metals in the aerial parts is higher than in the roots; however, in non-hyperaccumulating plants, heavy metals are usually not transported to the shoot, and the highest concentration is usually found in the roots. *Achillea millefolium* L. is a plant that has been characterized as a hyperaccumulator of heavy metals (Riyazuddin et al., 2021; Nikolova et al., 2018; Radanović et al., 2001).

The name "hyperaccumulator" describes numerous plants that are characterized by the ability to grow on soils rich in metals and the ability to accumulate extremely large amounts of heavy metals in shoots, far above the level in which they are found in most species, without phytotoxic effects. Three basic characteristics distinguish hyperaccumulators from related non-hyperaccumulator species: a markedly increased degree of absorption of heavy metals, faster translocation from roots to shoots, and a greater ability to detoxify and extract heavy metals in the leaves. The herbaceous perennial plant *Achillea millefolium* belongs to the *Asteraceae* family and is popularly known as yarrow. The genus *Achillea* includes over 130 perennial plant species native to the northern hemisphere from Europe to Asia and grows in temperate climates in arid or semi-arid habitats. Numerous studies done worldwide have found the plants of the *Asteraceae* family have strong antioxidant properties, antidiabetic activity, antimicrobial activity, anti-cancer activity, antifungal activity, antiviral and antihelminths activity. Because of their extremely complex chemical composition, species of the *Asteraceae* family show the many activities indicated. High levels of fiber and inulin, a complex carbohydrate, are characteristics of plants in the *Asteraceae* family. They also contain a variety of phenolic compounds, primarily phenolic acids, flavonols, anthocyanins, and sesquiterpene lactones, as well as important dietary phytochemicals, primarily carotenoids, tocopherols, and ascorbic acid. (Yanakieva et al., 2023; Kostić et al., 2020). Kostić et al., (2020). mentioned that more than 80 species belonging to the *Asteraceae* family are used extensively as edible plants in the Mediterranean and Balkan regions. Balijagić et al., 2021, mentioned that in Montenegro close to 300 plants are used in the pharmaceutical industry and in folk medicine; one of them *is Achillea millefolium. Achillea millefolium*, the best known and most widespread species, has been included among the most commonly used plant species in folk and conventional medicine for more than 3000 years (Mitich, 1990). *Achillea millefolium* is a perennial plant growing from 30 cm to 60 cm, with a single or sometimes forked stem. The leaves are from 5 cm to 20 cm long, finely pinnate, and the tips of the leaves end in tiny, prickly tips. The flowers are usually white and arranged at the top of the stem (Ali et al., 2017).

The content of heavy metals in plants helps us to assess environmental pollution. Metals such as iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), and chromium (Cr) are essential nutrients for plants, which are toxic only in high concentrations. Heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) are toxic metals that have no functional role in metabolism. One of the most serious environmental problems is the increasing presence of heavy metals in the environment, which causes plants to absorb them either from the soil or from the atmosphere through dry and wet deposition. This is associated with unsuitable agricultural practices, such as using soils that are heavily contaminated with metals or using excessive amounts of pesticides, herbicides, and fertilizers. Plants can further transfer the accumulated metals from the soil to the food chain (soil → plant → food), causing adverse effects on human health (Popescu et al., 2021). Many studies have been done on the chemical composition of *Achillea millefolium*, but in Bosnia and Herzegovina there is no systematic study of this plant considering its possible use in pharmacy, traditional medicine, and also for soil bioremediation. (Manojlović and Singh, 2012; Murtić et al., 2019; Murtić et al., 2021;

Based on everything previously mentioned, the aims of this research are: a) to determine the content of heavy metals in the leaves of *Achillea millefolium* sampled at five urban and five rural locations in Bosnia and Herzegovina; b) determine the content of heavy metals in tea infusions prepared from *Achillea millefolium;* c) to assess the level of environmental pollution with heavy metals and assess the exposure of the human body to heavy metals due to the consumption of tea infusion.

MATERIAL AND METHODS

Achillea millefolium samples were collected in October 2022 and May 2023 at ten selected locations in Bosnia and Herzegovina, including urban and rural areas. Sampling locations are shown in Figure 1. Fresh leaves of *Achillea millefolium* were collected during stable weather conditions, sunny weather, and no precipitation. About 500 g of the sample was collected in paper bags in order to prevent their contamination. The samples were cleaned of mechanical contamination, they were not washed so that the particles deposited on the surface of the leaf could be analyzed. The samples were dried in air and in a ventilated place for 3 weeks. The dried samples were crushed in the laboratory in a mortar using a pestle. About 1.0000 g (±0.0001 g) of the sample was weighed on an analytical balance, and all analysis was done in triplicate. Heavy metals were extracted from plant samples by acid digestion: 10 ml of 65% HNO3 was added to the sample, and it was heated at a moderate temperature until the evaporation of brown vapors stopped. After cooling to room temperature, 2.5 ml of 30% H2O2 was added, and heating was continued at the same temperature range until the solution became clear. The cooled solutions were filtered into a measuring vessel of 50 mL and diluted with ultrapure water (Figure 2).

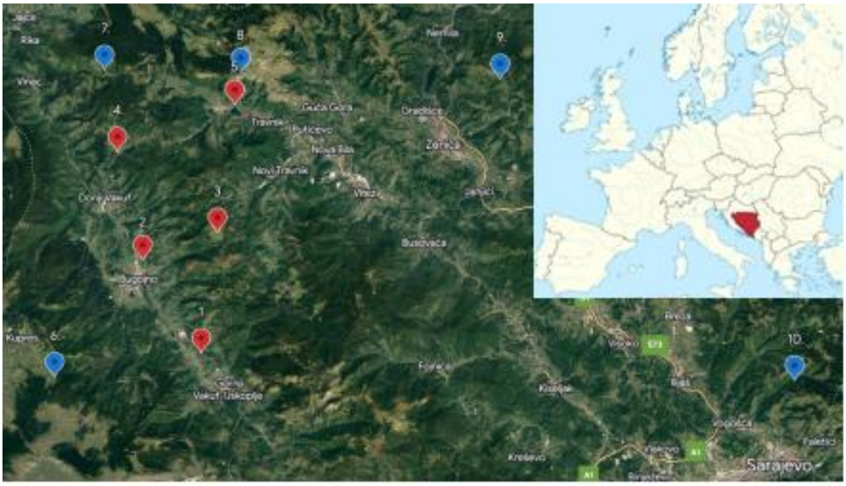


Figure 1. *Achillea millefolium* sampling location (Google Earth). Urban locations are marked in red, and rural locations in blue.

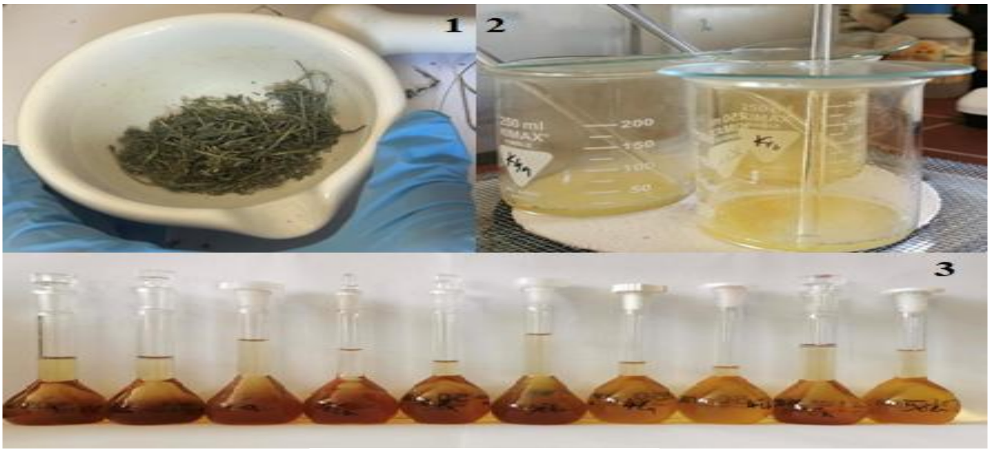


Figure 2. Sample preparation.
1-*Achillea millefolium*, 2-acid digestion, 3-sample solutions prepared for analysis

The prepared solutions were transferred into sterile plastic vials and stored at room temperature until analysis. The metal analysis was performed by using a flame atomic absorption spectrometer (FAAS), model AA240FS, Varian.

For the preparation of tea infusions, about 2.0000 g (± 0.0001 mg) of *Achillea millefolium* leaf sample was weighed on an analytical balance. Tea infusions were prepared according to the following procedure: 50 ml of ultrapure water was heated in laboratory beakers on a stove to the boiling point, then 2 g of the plant sample was added and allowed to boil for another 5 minutes with stands the plant sample was added and allowed to boil for another 5 minutes with occasional stirring. The cooled mixtures were filtered through qualitative filter paper into measuring vessels of 50 ml and diluted to the mark with ultrapure water.

Analytical quality control

All substances used were of analytical grade and supplied from Merck (Darmstadt, Germany). To ensure accurate heavy metal assessment, leaf and water infusion samples were spiked with the standard solution of each metal at three different levels of concentration to cover the measurement range. Recovery values are presented in Table 1.

Table 1. Recovery values of spiked leaf and tea water infusions of the *Achillea millefolium*

Metals	Recovery (%)	
	Leaves samples	Water infusion
Cu	96	96
Mn	98	99
Fe	96	95
Co	88	86
Ni	89	90
Cd	95	93
Pb	98	97
Zn	103	101

The recovery was calculated and ranged from 86% to 103% for all metals. The value of the detection limit (LOD) (calculated as three times the standard deviation of the blank signal) were: Cd (0.001 mg/L), Cr (0.006 mg/L), Cu (0.05 mg/L), Fe (6.15 mg/L), Mn (0.20 mg/L), Ni (0.015 mg/L), Pb (1.00 mg/L), and Zn (0.65 mg/L).

RESULTS AND DISCUSSION

Results of metal content (mean value ± standard deviation) in the leaf of *Achillea millefolium* sampled from the urban location are shown in Table 2, and in the Table 3 are shown results for metal content in the leaf sampled in the rural locations.

Results presented in Table 2. showed that the metal content in leaves of the *Achillea millefolium* sampled in all urban locations was arranged as the following diminishing series: Fe > Mn > Cu > Zn > Pb > Co > Ni, except from the urban location 5 (Turbe, Travnik), where diminishing serires were: Fe > Mn > Zn > Cu > Pb > Co > Ni. At the rural location, metal content in leaves of the *Achillea millefolium* varied from location to location. In rural locations 7 (Dogani, Jajce) and 8 (Vlašić-Galica), metal content was in the following diminishing series: Mn > Fe > Zn > Cu > Pb > Co > Ni. At location 6 (Kukavice, Kupres), the diminishing series: Mn > Pb > Fe > Cu > Zn > Co > Ni, while at location 9 (Smetovi, Zenica), it was: Fe > Mn > Zn > Cu > Pb > Co > Ni, and at location 10 (Nahorevska Brda, Sarajevo), it was: Fe > Mn > Cu > Zn > Pb > Co > Ni.

Table 2. Metal content in *Achillea millefolium* leaves sampled at urban location

Locations	**Metal content (mg/kg)**						
	Cu	**Mn**	**Fe**	**Co**	**Ni**	**Pb**	**Zn**
1	10.14 ± 0.36	68.47 ± 1.12	88.95 ± 8.61	0.132 ± 0.11	<LOD*	1.60 ± 0.45	6.30 ± 1.05
2	9.16 ± 0.05	73.63 ± 1.70	143 ± 19.54	<LOD*	<LOD*	0.684 ± 0.27	8.46 ± 0.68
3	7.42 ± 0.73	46.16 ± 1.13	82.14 ± 5	0.077 ± 0.06	<LOD*	2.59 ± 1.11	4.47 ± 2.53
4	9.70 ± 0.19	100 ± 1.76	138 ± 9.08	<LOD*	<LOD*	3.92 ± 1.46	5.98 ± 0.89
5	7.91 ± 0.01	52.88 ± 0.13	93.42 ± 4.64	<LOD*	<LOD*	1.22 ± 0.65	8.79 ± 0.96

<LOD* below the limit detection of the applied method

1-Pajić Polje, Gornji Vakuf; 2- Bugojno; 3-Rostovo; 4-Oborci, Donji Vakuf; 5-Turbe, Travnik

Table 3. Metal content in *Achillea millefolium* leaves sampled at rural location

Locations	**Metal content (mg/kg)**						
	Cu	**Mn**	**Fe**	**Co**	**Ni**	**Pb**	**Zn**
6	11.42 ± 1.04	78.50 ± 1.63	75.10 ± 4.73	<LOD*	<LOD*	77.72 ± 63.17	7.43 ± 1.12
7	7.76 ± 0.08	203 ± 7.35	52.14 ± 2.48	<LOD*	<LOD*	1.24 ± 0.23	24 ± 0.29
8	5.84 ± 0.11	82.72 ± 3.62	78.53 ± 8.17	<LOD*	<LOD*	4.12 ± 0.15	14.73 ± 1.52
9	11.16 ± 0.77	62.54 ± 0.15	230 ± 83.50	0.435 ± 0.01	<LOD*	2.46 ± 0.02	24.76 ± 15.86
10	11.54 ± 0.25	131.7 ± 0.95	245.7 ± 2.60	0.335 ± 0.27	<LOD*	3.32 ± 1.56	10.46 ± 2

<LOD* below the limit detection of the applied method

6- Kukavice, Kupres; 7- Dogani, Jajce; 8- Vlašić-Galica, Travnik; 9- Smetovi, Zenica; 10- Nahorevska Brda, Sarajevo

Based on the results presented in Table 2. and Table 3, the highest Cu content was recorded in three rural locations: Nahorevska Brda (Sarajevo), Kukavice (Kupres), and Smetovi (Zenica). Nahorevska Brda and Kukavice are rural areas where the population is mainly engaged in agriculture and animal husbandry, so the content of Cu can be explained by the use of fertilizers and pesticides, which represent an important anthropogenic source of Cu in the soil. Also, potential industrial and agricultural sources of Cu are batteries, pigments and dyes, alloys, fuels, and catalysts (Panagos et al., 2018; Rehman et al., 2019). On the other hand, Smetovi is a mountain located in the immediate vicinity of Zenica, a city known for its metal and metal processing industry. The ecosystems of the Zenica region have been exposed to the intense influence of heavy metals emitted from metallurgical plants for several decades. This affected the anthropogenic redistribution of heavy metals in the soil of the Zenica region. The soil and plants were heavily contaminated with heavy metals. However, the key metallurgical plants have not been operating since the beginning of 1992, which is why the soil load with heavy metals in that region has been greatly reduced (Goletić, 2003). Kabata-Pendias (2000) mentioned that the range of Cu content in plants from 5 mg/kg to 20 mg/kg is normal and necessary for proper growth and development of plants, while symptoms of Cu toxicity occur when the content exceeds the value of 30 mg/kg. The Cu content in this study at all sampling locations was within the range considered normal and sufficient for proper plant growth and development.

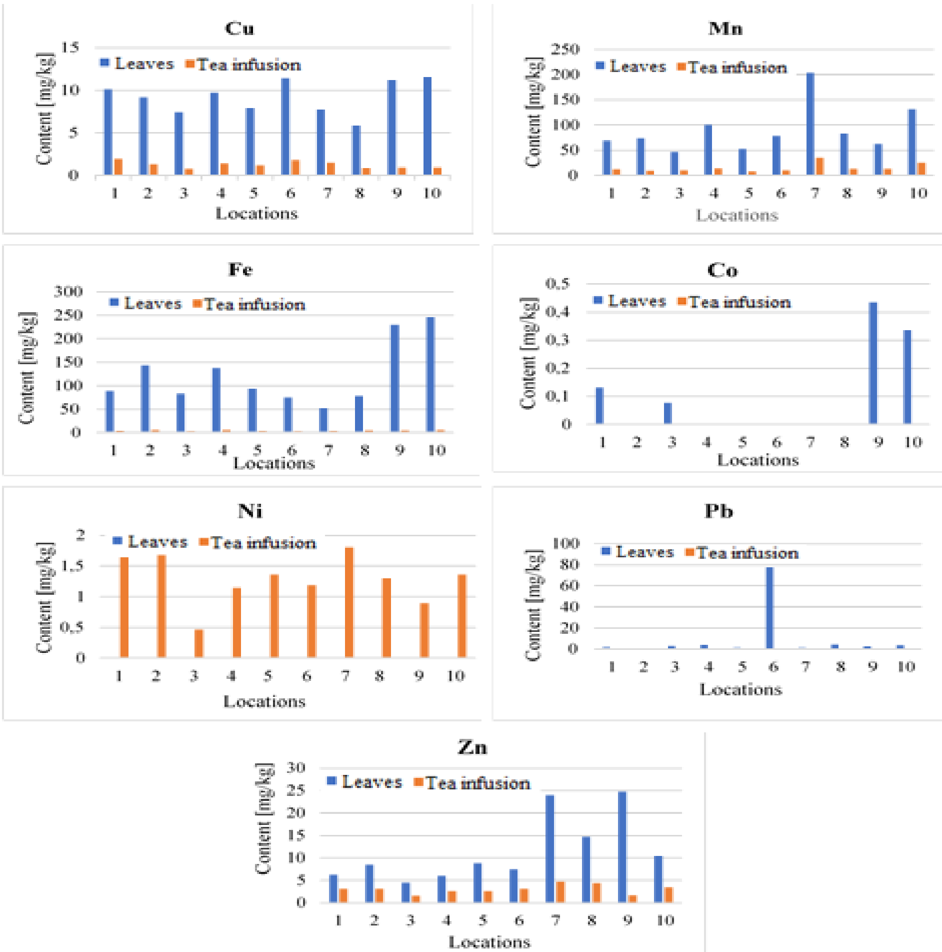
Kabata-Pendias (2000) mentioned that the normal range of Mn content in the leaves of medicinal plants is from 20 mg/kg to 300 mg/kg. Mn content at all sampling locations was within the specified normal range. However, a particularly high content compared to other locations was detected in the samples collected in Dogani, a village about 15 kilometers from the city Jajce. Namely, the factor that most determines the availability of Mn to plants is soil acidity; the more acidic the soil, the higher the availability of Mn. Soil analysis was not done in this experiment, but sampling at this location was done near wild blueberries (Vaccinium myrtillus), which are known to be adapted to acidic soil conditions and can only be found as wild species on such soil (Kabata-Pendias, 2000; Duralija and Konjević, 2022). Papludis et al., (2018) mentioned that Mn can enter the environment through the discharge of municipal waste water, waste sludge, mining and processing of mineral raw materials (smelting of metal ores), emissions during the production of steel and iron alloys, combustion of fossil fuels, and emissions during the combustion of fuel additives. Chizzola (2012) states that the critical value of Fe content below which deficiency symptoms can occur in plants is 50 mg/kg. On the other hand, the Fe content in the leaves is considered normal, even up to 2000 mg/kg. Due to the low toxicity of Fe, these high levels are not of concern to human health. Iron also has an important function in chlorophyll synthesis in the leaf (Živkov-Baloš et al., 2014).As can be seen in Table 2 and Table 3, the values of Fe content at all sampling locations are within the mentioned normal range. However, the highest Fe content was recorded on Nahorevska Brda and Smetovi. The high content of Fe in Nahorevska Brda can be explained by the fact that it is a rural area where fungicides and fertilizers are widely used, which are an important anthropogenic source of Fe in addition to mining and the metal industry. On the other hand, the high Fe content in Smetovi is explained by the fact that the mountain is located near Zenica, a city where steel production is still present, although to a lesser extent than before.

The content of Co in three urban locations (Bugojno, Oborci - Donji Vakuf, and Turbe - Travnik) and three rural locations (Kukavice - Kupres, Dogani – Jajce, and Vlašić - Travnik) was below the detection limit. Maiti (2003) states that the normal content of Co in plants is up to 1 mg/kg, while the range of critical content is between 15 mg/kg and 50 mg/kg. The Co content in this study was normal in all urban and rural locations where *Achillea millefolium* was sampled. According to the World Health Organization (WHO), the maximum permissible content of Pb in dried plant material is 10 mg/kg (WHO, 1998). The content of Pb in the leaf of *Achillea millefolium* was below the maximum permissible content according to WHO at nine out of ten sampling locations. An exception is the location of Kukavice, a village located about 5 kilometers from the center of Kupres, where a surprisingly high content of Pb was detected, almost eight times higher than the maximum allowed content. The reason for such a high concentration of Pb may be that there is a wood, textile, and metal industry in the sub-area of Kupres, as well as the fact that it is a rural location where Pb can originate from coal burning, car exhaust, mineral fertilizers, and municipal waste disposal sites.

The higher content of Zn in the leaves of *Achillea millefolium* was in the samples sampled from the locations Smetovi (Zenica) and in Dogani (Jajce). Kaur et al., (2014) state that one of the most important anthropogenic sources of zinc is steel production facilities. Given that the location of Smetovi is located near the city of Zenica, where there are steel production facilities, this could be one of the reasons for the increased zinc content in the samples of the leaves of *Achillea millefolium*. On the other hand, the higher content of Zn detected in Dogani is explained by the previously mentioned fact that it was caused by acidic soil. Kisić (2012) states that a higher Zn content can be expected on acidic soil. Maiti (2003) states that the critical content of Zn in plants at which symptoms of toxicity may occur ranges from 100 mg/kg to 400 mg/kg. Based on the results presented in Table 2 and Table 3, the Zn content in *Achillea millefolium* leaves was normal and far below the critical level at all sampling locations.

In Bosnia and Herzegovina, the Rulebook on the Maximum Permitted Amounts of Contaminants in Food does not prescribe the permitted amount of any tested metal in the tea infusion (Službeni glasnik BiH 37/09, 2009). On the other hand, in Croatia, the Ordinance on toxins, metals, metalloids, and other harmful substances that can be found in food prescribes a maximum permitted amount of Pb in domestic tea, which is 5 mg/kg (Narodne novine, 2005). The results of this study were compared with other studies (Diaconu et al., 2012; Gogoasa et al., 2013; Kočevar Glavač et al., 2017; Pehoiu et al., 2020; Popescu et al., 2021; Đelić et al., 2021), and the metal content was in the same or similar range.

One of the aims of the study was to determine the metal content in the tea infusion prepared from the leaves of *Achillea millefolium*. Figure 3 presents the results of the comparison of metal content in tea infusion and in the leaves of the *Achillea millefolium*.



* Note: 1 – Pajić Polje, Gornji Vakuf; 2 – Bugojno; 3 – Rostovo; 4 – Oborci, Donji Vakuf; 5 – Turbe, Travnik; 6 – Kukavice, Kupres; 7 – Dogani, Jajce; 8 – Vlašić-Galica, Travnik; 9 – Smetovi, Zenica; 10 – Nahorevska Brda, Sarajevo

Figure 3. Metal content in leaves and tea infusion of *Achillea millefolium*

By comparing the metal content in the leaves and the tea infusion of *Achillea millefolium*, it can be seen that the metal content at all sampling locations detected in the leaves was several times higher compared to the metal content in the tea infusion. The proportion of metals present in the leaf *of Achillea millefolium* that was soluble in water could be extracted by preparing tea infusions. The content of metals extracted in this way decreased in the following sequence: Mn>Fe>Zn>Cu>Ni>Pb.

As already mentioned, the content of Pb in the leaf of *Achillea millefolium* at the Kukavica location near Kupres was surprisingly high. By preparing a tea infusion from *Achillea millefolium* from the same location, an extremely low content of Pb (0.224 mg/kg) was detected, which did not significantly differ from the content of this metal in tea infusions from other locations. This tells us that Pb is present in this plant mostly in forms that are not soluble in water. The content of Co in all samples of tea infusion was below the detection limit of the applied method, while in the leaf of *Achillea millefolium* Co was detected in samples from four out of ten locations (Pajić Polje - Gornji Vakuf, Rostovo, Smetovi - Zenica, Nahorevska Brda, Sarajevo). On the other hand, Ni was detected in the tea infusion, but its content in the *Achillea millefolium* leaves itself was below the detection limit of the applied method. This phenomenon could have occurred for several potential reasons: (1) it is possible that Ni in the leaf of *Achillea millefolium* is present in a form that is more soluble in water than in nitric acid; (2) it is possible that Ni formed complex compounds with some of the components released by the decomposition of plant material during the acid digestion procedure, which made it impossible to detect it in the leaves of *Achillea millefolium* using FAAS. Results from the studies: Dobrinas et al., (2011); Diaconu et al., (2012); Altıntıg et al., (2014) and Popović et al., (2017).

CONCLUSIONS

The metal content in the leaves of *Achillea millefolium* depends on the place where the sampling was carried out and the potential natural or anthropogenic sources of heavy metals located nearby, as well as on the acidity of the soil. The metal content in tea infusions depends on the solubility in water of the particles found on the surface of the leaves or from the chemical form in which the metal is absorbed inside the leaves of *Achillea millefolium.*

Future research related to the preparation of the tea infusion and the amount of extracted metals could be related to the assessment of the influence of the shredding of the leaves, the amount of dry or raw sample, the length of the extraction time, as well as the use of different parts of the plant (root, stem, leaf, and flower).

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VALUATION OF AGRICULTURAL LAND IN RELATION TO LOCATION OF CITY CENTERS AS A FACTOR IN THE MANAGEMENT OF MENTIONED RESOURCE

SUMMARY

Observing the value of agricultural plots is one of the important factors in organizing agricultural production in an economy. The main conclusion of the author would be that there are significant differences (based on the t-test) in the value of agricultural plots, i.e. plots are more expensive if they are located up to 25 km from the center of large cities compared to remote plots that are at a greater distance than 25 km. Secondly, the value of agricultural plots is higher for agricultural plots that are located closer to the center of large cities, as well as the fact that the main reason for buying such plots is their further sale, while the reason for buying more distant plots is the continuation of agricultural production, which is valid for the entire observation period of 2022-2024. Third, there is no significant connection between the value of agricultural land and the reasons for purchasing agricultural land for 2022 and 2023, as well as the fact that the purchase of agricultural plots is rarely carried out in order to consolidate agricultural holdings.

Key words: value of agricultural land, reason for land purchase, management, business results.

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INTRODUCTION

The organization of agricultural production largely depends on realistic representations of the values of all factors that can influence the correct and optimal way of management, as pointed out in numerous works by authors such as (Turak & Koop, 2003; Aničić & Popović, 2015; Vitomir et al., 2020; Astuti et al., 2022).

One of the important factors that should be paid close attention to is the factor of real valuation of agricultural land as part of observing the real organization of agricultural production, which we see in the already published works of authors (Baen, 1997; Abraham, 2020; Zhu et al., 2021; Chen et al., 2022).

Observing the organization of agricultural production is a complex issue, where above all the value of agricultural land represents the first step that affects the formation of clear business decisions by decision makers that are essential for optimal business in agriculture (Wadduwage, 2021; Komariah & Matsumoto, 2021; Schmitt et al., 2022; Biratu et al., 2023; Vinogradovs et al., 2023; Hussain et al., 2024).

However, in addition to the valuation of agricultural land, the existence of other factors that have an impact on the optimization of agricultural production should be taken into account, which should be considered as a basis for making valid management decisions as a whole, which are related to the normal functioning of agriculture in an economy (Munitlak et al., 2015; Eludoyin & Adewole, 2020; Willkomm et al., 2021). The existence of other influences in terms of the organization of agricultural production was pointed out by other authors, each from their own point of view, such as (Matsui et al., 2021) focus on land, authors such as (Siba et al., 2022) focus on climatic influences, authors such as (Yazdandoost et al., 2022) focus on the influence of the water regime and others. Apart from such an observation, there are also authors who researched the application of innovative achievements that can be applied in the organization of agriculture, which was pointed out by authors such as (Dragosavac et al., 2023; Sodjinou, 2024).

In addition, the organization of agricultural production requires a complex multidisciplinary approach using numerous factors (Hojjatnooghi et al., 2019; Grau et al., 2020; Daniels et al., 2023; Bojović et al., 2019; 2024; Popović et al., 2024a; 2024b; Golijan Pantović et al., 2024). However, in a large number of studies, authors draw attention to essential issues related to the physical properties of the soil, such as authors (Chumakov et al., 2024) who focus on afforestation of areas, authors who point to the complexity of the biosphere on agriculture (Fida et al., 2024), in relation to the importance of irrigation, such as authors (Islam et al., 2024).

Making valid management decisions related to the valuation of agricultural land, but also other factors that can affect business results are present in numerous activities, noting that it is of great importance for management within the organization of agricultural production in countries that want to accelerate are developing and who want to have security in the provision of agricultural products for their population (Jokić et al., 2020; Arnautović et al., 2022; Kockelkoren et al., 2023; Moisa et al., 2023).

The assessment of land value is often positively associated with the implementation of measures by state authorities, such as land consolidation measures, the application of which can increase the market value of agricultural plots, depends on state measures to encourage the purchase of agricultural plots by subsidizing, for example, certain groups such as "young farmers", with full respect for the legal provisions that accompany the circulation of agricultural land in the Republic of Serbia. However, all these measures should be viewed as part of the real valuation of agricultural plots in order to realistically view the movement of the value of agricultural land in a given economy.

Land valuation is followed by trends in the land market, which was the starting point for this study in order to practically analyze trends in the increase in the value of agricultural plots that are closer to large cities, so that the economics of valuation often have an advantage over the organization of agricultural production.

MATERIAL AND METHODS

The focus of the authors of this study was aimed at observing the value of agricultural plots that were located at different distances from the center of the three largest cities in the republic of Serbia, namely in Belgrade, Novi Sad and Niš. The distance was observed through two criteria up to 25 km distance from the center of the mentioned cities and at a distance of more than 25 km. In addition to that basic observation, the research was deepened to observe another factor influencing the valuation of agricultural plots, more precisely the reasons why the respondents decided to buy agricultural plots depending on the distance from the center of the mentioned cities, looking at the possibilities for the decision of the respondents through: continuation of agricultural production, consolidating agricultural land and selling it in order to make a profit on agricultural land.

All this was done by the author in the following way. The survey was conducted in a three-year observation period, more precisely in the period from 2022 to 2024 and from 01.03 to 20.03. The mentioned years. All participants are guaranteed anonymity, as well as the fact that their generals will not be used and disclosed, but that they will be used only for scientific purposes, that is, for the preparation of this study 178 participants were surveyed in each of the observation years 70 participants were surveyed each year whose plots were evaluated using the criteria of being located at a distance of up to 25 km from the center of large cities, as well as 108 respondents whose plots were at a greater distance than 25 km.

The essential choice of distance from the center of large cities was made on the basis of interviews with 10 real estate agencies in all three large cities that were the subject of the research, interviews with five law firms in all three cities and personal visits to 10 locations at both distances in the three cities. The authors wanted to obtain representative and as realistic data as possible so that the study would have practical value in addition to theoretical value. The same respondents were surveyed in all three years of the research and included about 90% of individual households and about 10% of companies that are mainly engaged in agricultural production. In the structure of agricultural production in the first circle of large cities, about 90% of agricultural producers were engaged in vegetable and berry production, while outside the first circle, i.e. more than 25 km from the city center, agricultural production of field and industrial field crops dominates with about 90%. The average purchase and sale of plots within a radius of 25 km from the city center was about 1.5 ha, and at a distance of more than 25 km it was 4.8 ha.

The second criterion or factor was an examination of the differences in the reasons why the plots were purchased by the respondents, with the possibility for the respondents to choose one of the answers: that the plots are intended for the continuation of agricultural production, consolidation of areas or for resale, depending on the proximity to the center the three largest cities in the country.

In order to realistically show the value of agricultural land, the data of law agencies were used, with the fact that the authors of the study rejected about 5% of values that deviated significantly (about 30%) in relation to the estimated value of agricultural land in that or a similar location, because it was an unrealistic representation due to the reduction of the value due to the reduction of the possible estimated sales tax on the value of the land, i.e. the authors used publicly available data from tax administrations of local self-governments as a corrector and after that, in order to realistically present the research, they eliminated such individual cases of valuation of agricultural plots.

When preparing this study, the authors did not use the valid (new) land categorization in the Republic of Serbia. Essentially, the division of land into: total used agricultural land, uncultivated agricultural land, uncultivated agricultural land in overgrowth, forest land and uncultivated land under buildings, roads, ponds, ponds and reeds is not included. The primary interest of the author was focused on the market value of land.

The participants of the survey in the largest number of cases in the evaluation of plots at a distance of up to 25 km from the center of large cities are mainly determined for the production of vegetables and berries, while the plots that are further than that 25 km from the center are mainly used for agricultural production and industrial production of sugar beets, sunflowers, soybeans and rapeseed.

The method of market assessment of the value of agricultural land was used with an exclusive focus on the location where the agricultural plot is located, specifically in relation to the center of three large cities in Relpublica Serbia. Thus, the authors were focused on realistic presentation of the subject of purchase and sale.

The authors put forward the following hypotheses:

H: 1 there are no significant individual differences in the value of agricultural land in relation to the distance from the center of large cities in the Republic of Serbia in 2022, 2023 and 2024, as well as the total observed.

H: 2 there are no significant individual differences in the reasons for purchasing agricultural land in relation to the distance of the plot from the city center in 2022, 2023 and 2024, as well as the total observed.

H: 3 that there are no significant relationships between the value of agricultural land and the reasons for purchasing agricultural land for plots up to 25 km from the center of large cities.

H: 4 that there are no significant correlations between the value of agricultural land and the reasons for purchasing agricultural land for plots more than 25 km from the center of large cities.

Statistical data processing and analysis were done using the software ibm SPSS (statistical package of social science) version 25. The t-test of independent samples was applied in the paper to examine the difference in land value and reason for purchase between plots in relation to proximity to the city center. Pearson's correlation analysis was applied to examine the association between land value and reason for purchase. A level of 0.05 was used for the threshold value of significance.

RESULTS AND DISCUSSION

The obtained research results are grouped into three units.

Within the first unit, the results showed trends in the value of agricultural plots in the research period 2022-2024 in relation to the location measured from the center of the analyzed cities.

In the second part, the reasons why the respondents decided to buy agricultural plots in the research period 2022-2024 are presented in relation to their position, i.e. the location in relation to the center of the analyzed cities.

In the third part of the research, the relationship between the value of agricultural land and the reasons for purchasing agricultural land for plots up to 25 km from the city center, as well as for plots located outside of 25 km from the city center, is presented.

Presentation of the results of the value of agricultural land in relation to the distance from the center of large cities in the Republic of Serbia

The presentation of the results of the valuation of agricultural land in relation to the distance from the center of large cities is given in Table 1.

The results indicate that there is a significant difference in the value of agricultural land observed in 2022, especially if the location of agricultural plots is analyzed, especially if they are located closer to the city center. Examination of differences was powered using an independent samples t test (Table 1). Based on this, the value of agricultural land is much higher for plots that are up to 25 km from the city center.

Table 1. Differences in the value of agricultural plots for 2022-2024 and the total observed for the entire observation period.

Year of observation	Plot distance up to 25 km from the city center	The plot is more than 25 km from the city center	t	p
	Middle value			
2022	11635.90 ± 820.67	6717. 25 ± 384.68	46.915	<0.0005*
2023	26785.85 ± 2751.11	8465.05 ± 6721.26	21.644	<0.0005*
2024	80241.10 ± 4009.99	9889.49 ± 2382.70	132.413	<0.0005*
Total period	39554.28 ± 1493.68	8357.26 ± 2313.65	109.320	<0.0005*

*Statistical level of significance at the level of 0.05
Source: authors' calculation (2024).

The results in the second year of observation related to the display of differences in the values of agricultural plots in relation to the distance from the city center. It can be seen that there are significant differences in the value of agricultural land, i.e. agricultural plots for the analyzed year 2023. In addition, after the t-test, it can be seen that there are values of agricultural land that differ significantly in their value in relation to their location in relation to the center of large cities. The value of agricultural plots is significantly higher for plots that are up to 25 km from the center of large cities compared to those agricultural plots that are more than 25 km from the center of large cities.

The results shown indicate the existence of significant differences in the value of agricultural land for 2024. After the t-test, it can be seen that the value of agricultural land (agricultural plots) is higher for the analyzed plots located up to 25 km from the center of large cities compared to those agricultural plots located further from the center of large cities.

The results show that there is a significant difference based on the value of agricultural plots for the entire observation period from 2022 to 2024 in relation to the proximity of the location to the center of large cities. The value of agricultural land in the entire observation period, i.e. from 2022 to 2024, is higher for the analyzed agricultural plots located up to 25 km from the center of large cities compared to those agricultural plots located at a greater distance than 25 km from the center of large cities, which is supported by the results obtained after the survey.

Respecting the setting H:1, it can be pointed out that the results of the research showed that there are significant differences in the value of agricultural plots in relation to the distance of the plots that are located from the center of large cities, and that in the entire observation period of 2022-2024. Therefore, the valuation of agricultural land depends on numerous internal factors related to the making of management decisions, which was pointed out in their works by authors such as (Popović et al., 2016; Lakić et al., 2024), which largely coincides with the obtained research results that indicated the existence of specific differences that may arise in the valuation of agricultural plots in relation to their location.

Presentation of the results of the value of agricultural land in relation to the examined differences in relation to making a decision on the purchase of agricultural land in relation to the distance from the center of large cities in the Republic of Serbia

The presentation of the obtained results is given in Table 2. Based on the results shown in Table 2, it can be seen that there is a significant difference based on the analyzed reasons for purchasing agricultural land for the year 2022 based on the established differences between plots in relation to the location from the center of large cities.

Table 2. Presentation of differences based on the reasons for purchasing agricultural land for 2022-2024 and overall.

Year of observation	Plot distance up to 25 km from the city center	The plot is more than 25 km from the city center	t	p
	Middle value			
2022	2.87 ± 0.41	1.16 ± 0.48	24.283	<0.0005*
2023	2.84 ± 0.47	1.24 ± 0.57	20.258	<0.0005*
2024	2.90 ± 0.38	1.24 ± 0.56	23.344	<0.0005*
Total period	2.87 ± 0.41	1.21 ± 0.52	23.482	<0.0005*

*Statistical level of significance at the level of 0.05
Source: authors' calculation (2024).

The obtained results are such that plots located closer to the center of large cities were mostly bought for further sale, and plots at a greater distance of 25 km from the city center were mostly bought for continued production.

The results indicate that there is a significant difference based on the reason for purchasing agricultural land for the year 2023. After the t-test, the results obtained (Table 2) are such that differences can be seen based on the reasons for purchasing agricultural plots in relation to the location near the center of large cities. Plots closer to the center of big cities were mostly bought for further sale, and plots at a greater distance of 25 km from the center of big cities were mostly bought for the continuation of agricultural production.

The results indicate that there is a significant difference based on the reasons for purchasing agricultural plots in 2024. The presentation of the obtained results in Table 2 refers to the determined differences between plots in relation to the proximity to the center of large cities. Plots closer to the city were mostly bought for further sale, and plots at a greater distance of 25 km from the city center were mostly bought for continued production.

The results indicate that there is a significant difference based on the reasons for purchasing agricultural land, which refer to the entire research period. The entire period of observation from 2022 to 2024 is such that a significant difference can be seen between agricultural plots in relation to the proximity of the center of large cities based on (factors) reasons for purchasing agricultural land (plots), where plots located closer to the city were predominantly purchased for further sale, and plots at a greater distance of 25 km from the center of large cities were mostly bought for the continuation of agricultural production.

In addition, taking into account the statement H: 2, we determined that there are significant differences regarding the formation of reasons for the purchase of agricultural land in relation to the observation of the distance of agricultural plots in relation to the center of large cities in the entire observation period of 2022-2024.

Presentation of the results of the connection between the value of agricultural land and the reasons for purchasing agricultural land in the observation period 2022-2024.

The results obtained based on the analysis of the connection between the factor of the value of agricultural land and the factor of reasons for purchasing agricultural land for the observation period from 2022 to 2024 are presented in Table 3.

Table 3. Correlation between the value of agricultural land and reasons for purchasing agricultural land for plots located up to 25 km from the center of large cities

Analyzed factor	Observation period	Reasons for buying agricultural plots
Value of agricultural plots	2022	0.129
	2023	0.008
	2024	-0.485*
	Total period	-0.464*

*Statistical level of significance at the level of 0.05

Source: authors' calculation (2024).

In addition, the obtained results were based on the use of Pearson's correlation analysis. Based on that, it can be seen that there is a significant connection between the value of agricultural land and the reasons for purchasing agricultural land for the year 2024 as well as for the entire observed period.

Starting from the already expressed views of the authors (Popović et al., 2015; Popović, 2018; Radović et al., 2021; Čolović et al., 2024) regarding the importance of proper analysis and heterogeneous factors on the value of agricultural land, the existence of complementarity with the results obtained in this study can be seen.

Below is a presentation of the obtained results, which are shown in Table 4. The presentation given in the last table indicates that there is no significant connection between the value of agricultural land and the analyzed reasons for the purchase of agricultural land for the years 2022, 2023, 2024, as well as for the entire observed period, which is confirmed by the obtained values, which are low values of the correlation coefficient.

Table 4. Correlation between the value of agricultural land and reasons for purchasing agricultural land for plots located more than 25 km from the center of large cities

Analyzed factor	Observation period	Reasons for buying agricultural plots
Value of agricultural plots	2022	-0.094
	2023	-0.055
	2024	0.068
	Total period	0.023

*Statistical level of significance at the level of 0.05
Source: authors' calculation (2024).

Analyzing and summarizing the research results, the authors point out that in their opinion the research fully justified its existence. In particular, they point out that it showed that there are significant differences in the field of agricultural land valuation on the one hand, as well as from the point of view of discovering the reasons for the purchase of agricultural land for the period from 2022 to 2024 in the Republic of Serbia. All of this was determined based on the research of movement in relation to the location of plots near the center of large cities.

The results we reached indicate a partial acceptance of hypothesis H: 3, that is, that there are significant connections between the value of agricultural land and the reason for purchasing agricultural land for agricultural plots located at a distance of up to 25 km from the city center.

It was found that H:4 can be rejected completely, that is, there is a significant connection between the value of agricultural land and the reason for purchasing agricultural land for plots located more than 25 km from the center of large cities and that in the entire observation period of 2022-2024. All of this was confirmed using Pearson's correlation analysis, that is, there is a connection between the middle and negative levels. The negative association indicates that as the value of land increases, the demand for buying land for sale increases. The results obtained in the study largely coincide with the already stated views of authors such as (Popović et al., 2017; Negassa et al., 2023; Saleem et al., 2024) regarding the creation conditions for the unhindered performance of agricultural production (Terzić et al., 2018; 2019; Stevanović et al., 2023; 2024; Kosev et al., 2023; 2024).

In addition, the study shows that there is no significant connection between the value of agricultural land and the reasons for purchasing agricultural land for 2022 and 2023, which is confirmed by the low values of the correlation coefficient for the observed continuation of agricultural production and the further sale of agricultural plots, as well as the fact that respondents rarely buy agricultural land plots so that the total agricultural holding in the observed period 2022-2024 regardless of the distance of agricultural plots in relation to the location in relation to the center of large cities.

The statements in tables 1-4 indicate that the value of agricultural plots is very important and in subsequent research it is possible to expand the research to other motives related to the purchase of agricultural plots, such as those that are intended for further sale, improvement of production, whether the focus is quick profit by reselling plots that are close to cities, etc.

The land consolidation process in the Republic of Serbia is a necessary applicable factor for agricultural producers, because the land consolidation process optimizes agricultural areas, which mainly occurs outside the area of 25 km from the center of large cities, where local governments are involved in land consolidation processes based on the Land Consolidation Law, through the practical engagement of surveyors, where they often fully cover the costs incurred as part of land consolidation, and the consolidation of plots mainly refers to the organization of plots in farming and the production of industrial crops. The framework for such consideration by state authorities is the implementation of the Law on Agricultural Land ("Official Gazette of the Republic of Serbia", No. 62/2006, 65/2008 - other law, 41/2009, 112/2015, 80/2017 and 95/2018 - other law), Regulation on the content, procedure of development and adoption of the consolidation program ("Official Gazette of Republic of Serbia", No. 62/2020).

In the following researches, it is possible to pay more attention, i.e. put in the focus of the researches the categorization of the land in relation to the real market valuation, with the fact that the results we obtained in the study show the importance of studying the proximity of agricultural land in relation to the center of large cities as a real indicator that must be taken into account in the case of a real expression of the value of agricultural land.

The application of this study is possible in most of the countries of the former Yugoslavia, taking care to adapt them to the actual state of the respective economy, that is, the state of the economic system, which has its own specificities. This study relies on the application of international accounting standards that are valid in the accounting of developed economies, that is, which is visible in works such as (Popović et al., 2017). Nevertheless, this study in the methodological sense is innovative and in a way it was applied for the first time and applied in this study, and in general it can be expanded in the future both in the research sense in the Republic of Serbia and also to all the countries of the Western Balkans.

CONCLUSIONS

The results of this study point to the importance of real consideration of two important factors, the application of which can influence the improvement of the management of the agrarian organization in one economy. Two factors were analyzed in the paper: the value of agricultural plots, as well as the factor of reasons for buying agricultural plots, which was analyzed in relation to the proximity of locations to the center of three large cities in the Republic of Serbia.

The first conclusion would be that there are significant differences in the value of agricultural plots in relation to the distance of the plot from the center of large cities in the entire observation period of 2022-2024. The value of agricultural plots is higher in the analyzed agricultural plots that are located up to

25 km in relation to their distance from the center of large cities compared to those agricultural plots that are located at a distance of more than 25 km, which is reinforced with the results obtained after the conducted t- test.

Another conclusion would be that there are significant differences regarding the formation of reasons for purchasing agricultural land in relation to the observation of the distance of agricultural plots in relation to the center of large cities in the entire observation period of 2022-2024. At the same time, plots located closer to the city were mostly bought for further sale, and plots at a greater distance of 25 km from the center of large cities were mostly bought for the continuation of agricultural production.

The third conclusion would be that there is no significant connection between the value of agricultural land and the reason for purchasing agricultural land for 2022 and 2023, which is confirmed by the low values of the correlation coefficient for the observed continuation of agricultural production as well as for the further sale of agricultural plots, as well as the fact that rarely purchases agricultural plots in order to consolidate agricultural holdings, which refers to the entire observed period of 2022-2024, regardless of the distance of the agricultural plots in relation to the distance of the location in relation to the center of large cities in the Republic of Serbia.

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NEMATODE COMMUNITY STRUCTURES DEPICT SOIL HEALTH STATUS OF KOLA NUT TREE (COLA SPP.) FORESTS OF SOUTH-WEST NIGERIA

SUMMARY

Nematodes, the most prevalent multicellular organism in soil, hold a crucial position in the construction of the soil food web. They have been established as being excellent indicators of soil conditions which ultimately influence crop productivity. A survey was conducted to evaluate the soil nematode assemblages of kola tree forests in Oke Geu, Litaye, Ajue and Oniparaga villages of Ondo state, Nigeria. Soil samples were taken at random from all fields. The nematodes encountered were identified to generic level. A sum of 27 nematode genera, spanning 20 families, were discovered in sampled kola fields. Eight genera of plant parasitic nematodes were recovered, *Helicotylenchus* and *Paratylenchus* were the most abundant species. In line with all previous reports of free-living nematode assemblages in Nigeria, this study established that bacterivores were the most common nematodes. No significant difference ($P < 0.05$) was observed across all indices of ecosystem measured except with respect to the mean plant parasitic index and PPI/MI ratios. According to the food web study, 25% of the samples were plotted in quadrat B, reflecting mature and nutrient-rich soil conditions based on their metabolic footprints, while the remaining 75% were lotted in quadrat C, indicative of stable and fertile soil. Results further emphasized the importance of utilizing the concept of nematode c-p values in interpreting food web status of different soil habitats. The findings from this research added to the body of knowledge already available on the application of nematodes as markers of soil health and

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furthermore provides the first study of nematode assemblages in kola tree fields in West and Sub-Saharan Africa.

Keywords: Nematodes, kola nut, ecosystem functions, soil food web, metabolic footprint

INTRODUCTION

Cola nitida and *Cola acuminata* (Kola) belongs to the family Sterculiacea. They are large robust trees that are commercially grown in tropical climates of the world (Hutchinson and Dalziels 1963; Asogwa et al. 2006; Onaolapo and Onaolapo 2019; Jacob et al. 2024). Several varieties abound, with over 40 species known but the most common and of paramount importance being *C. nitida* and *C. acuminata* (Daramola, 1978; Oladokun, 1982; Odutayo et al. 2018; Jacob et al. 2024). They are generally found along west African coast, occurring predominantly in the Ghanaian and Ivorian forests (Hutchinson and Dalziels 1963; Opeke, 2005). The nuts could either be white, red or pink in colour (Nkemakolam, 2002; Nyadanu et al. 2020; Ichetaonye et al. 2024). Kola nuts are considered as a principal trade item to a great extent in west Africa and Nigeria for centuries (Mokwunye, 2009; Adesida et al. 2021). Nigeria produces up to 88% of total world kola nut, with 200,000 metric tonnes annually (Atanda et al. 2011; Oladigbolu et al. 2023). A multitude of Africans treasure and regard kola nuts symbolically in custom, tradition and heritage, because it exemplifies cordiality, bond and amity thus it is consumed at reunions, social and religious gatherings and more (Asogwa et al. 2012; Unya et al. 2021).

Scientifically, several medicinal merits are identified with kola nuts. It contains phytochemical compounds such as phenolics, theophylline, tannins, theobromine betanine and principally caffeine which is in a very high concentration. Kola nuts have been indicated in the treatment of some health challenges like diarrhoea, asthma, malaria, whooping cough, nausea and toothache (Obineche, 2017; Blades, 2000). It may be taken as a stimulant, poison antidote and tranquilizer (Adebayo and Oladele 2012; Aniwada and Ezema 2022). It serves as an excellent stimulant and eliminates fatigue. Kola nut pod husk is an important by product of nut processing with high nutritional value, it is employed as a feed additive in ruminants and poultry (Babatunde and Hamzat 2005; Lateef, 2023). Kola nut is also of high significance in pharmaceutical, textile, food, and beverage industries (Ogutuga, 1975; Olunloyo, 1979; Jaiyeola, 2001; Lateef, 2023; Ichetaonye et al. 2024). Kola nut trade is a thriving business in many local markets in Africa, where bulk trading of the nuts is directed to long distances as wholesale (Adesida et al. 2021). The recent scarcity of kola nuts which was occasioned by decline in yield observed within the last 12 years has been attributed to several factors such as increase in the number of unfruitful and sterile trees which was further linked to attack by field pests, pathogens, aging trees, reduced pollination and in some cases, poor soil conditions (Moshood, 2020; Azeez, 2021; Bello et al. 2022a).

Nematodes are known to be the most abundant multicellular organism in the soil found occupying the second, third and fourth trophic levels in the soil food web structure (Du Preez et al. 2022). They have been suggested as being excellent indicators of soil conditions which ultimately influence crop productivity (Ferris, 2010). Owing to their significant role in the soil food web, nematodes have been used to assess soil conditions in situ by evaluating their dynamics and interpreting their community structures (Young and Unc 2023). The integration of nematode feeding habits is represented by functional guilds (Yeates et al. 1993).

Furthermore, colonizer-persister (C-P) scales Bongers and Bongers (1998) was developed for studying faunal food web analysis while metabolic footprints were instituted (Ferris, 2010) for a more holistic study of soil health community dynamics based on nematode life strategies.

Bongers (1990) grouped nematodes on a continuum (r - to k- strategies) where r-strategies being the colonizers are those sensitive nematodes that occupy nutrient rich habitats and lay numerous small eggs. In contrast, during temporary circumstances of increased food supply, the k-strategies (persisters) scarcely react. According to this categorization, nematodes are divided into five groups on the C-P scale: carnivore nematodes, fungi feeders, omnivore nematodes, bacterivores and plant feeders. Moreover, Bongers and Bongers (1998) introduced the idea of functional guilds in an effort to incorporate nematode feeding groups into the life strategy principles. This comprises of species with comparable growth, metabolic, and reproductive traits and serving similar ecological roles. While the ecological significance and worth of soil nematodes have been acknowledged since the early 1960s (Banage, 1963), their role in controlling the densities of soil bacteria and fungi brought them to the forefront of the soil nutrient cycling process (Perez-Moreno and Read 2001; Hoorman, 2011). This gave rise to a more optimistic understanding of nematodes' functions in soil processes (Yeates, 2003). The dynamics of nematode communities and their significance have been researched on a variety of crops throughout the world (Schorpp and Schrader 2017; Tian *et al*. 2020). However, very little is known about abundance, distribution, and ecological services they offer within the agro-ecological systems of sub-Saharan Africa (SSA).

There are reports on several field and storage pests of kola nut in literature. However, despite the importance of this crop, information on its associated nematodes (both free living and plant parasitic) is sparse, hence the need for this study. Using kola tree fields as a case study, the current study looked at these factors in order to provide a good knowledge of how nematode community structures relate to soil health dynamics. Making decisions about soil conservation that will help SSA achieve stable and sustainable forest soil health depends on this understanding.

MATERIALS AND METHODS

Nematode sampling

Four farms on an expanse of 24 hectares (6 hectares each) located at Oke Geu, Ajue, Oniparaga and Litaye villages in Ondo state, Nigeria were sampled. An average of 820 kola nut trees in each farm was sampled separately between July and November, year 2021. The field on each farm was divided into rows and columns for easy sampling and adopting the systemic method of sampling. The top soil around each tree was scrapped to remove weeds and other particles. 820 individual samples were collected from the field. Soil samples were taken around the rhizosphere of each tree at a distance of 60 cm from the tree stem. Four points were sampled around each tree at a depth of 20 cm with a hand trowel. Samples from each tree was mixed to represent the particular tree. Each soil sample weighing approximately 1500 g was packed with label and then moved to the laboratory for evaluation. In the laboratory, the soil in each of the samples were thoroughly mixed and 200 cm^3 was taken out for nematode extraction by sieving and decanting following the method of Cobb (1918).

Nematode extraction, counting and identification

Composite soil samples were mixed and 200 cc sub-sample was taken from each and used for extraction. Nematode extraction from the sub-samples followed a modified sugar floatation technique (Jenkins, 1964). The recovered nematodes were placed in a Doncaster counting dish (Doncaster, 1962). Following procedure of Nico et al. (2002) 4% formaldehyde solution was introduced. Nematodes were identified by mounting them on glass microscopic slides with anhydrous glycerin. The University of Nebraska-Lincoln UNL Nematology Laboratory's interactive diagnostic keys were used in addition to the pictorial keys created by Jairaipuri and Ahmed (1992), Andrassy (2005), and Holovachov et al. (2009).

Frequency of occurrence and mean nematode abundance were expressed per field and collectively by calculating the Prominence values (PV) for each nematode genus identified according to DeWaele and Jordaan (1988) using the equation:

$$PV = \text{Population density } X \sqrt{\text{frequency of occurrence}} /10$$

Nematode diversity was expressed according to nematode feeding groups and their C-P scale values. Soil faunal (food web) structure was calculated according to Ferris et al. (2001; 2004). The structural, basal and enrichment indices trajectories were obtained from nematode abundance in guilds. The following equation was used in their calculations: Kbnb, where nb is the abundance of nematodes in each guild and kb is the weightings given to the guilds to indicate the features of the food web. Additionally, the following formulas were used to generate the structural indices (SI) and enrichment indices (EI):

$$100x(e/(e+b)) \text{ and } 100x(s/(s+b)), \text{ respectively.}$$

The Nematode Indicator Joint Analysis (NINJA) software programme was used to determine the total biomass and metabolic footprints (Sieriebriennikov et al. 2014). Lastly, based on the nematode faunal composition of each field mapped in the faunal profile, the soil condition of each field sampled was categorized into quadrats A, B, C, and D (Ferris *et al*. 2001; 2004).

RESULTS AND DISCUSSION

Nematodes from all trophic levels (Plant feeders (PPNs), Bacterivores, Fungivores, Omnivores and Predators) were recovered from soils of kola tree fields sampled. A total of 27 genera from 20 families of nematodes were recovered from soil samples (Table 1; Fig 1). The most prevalent groups were omnivores (six genera), the predators (three genera), the fungivore, bacterivores and PPNs (each with eight genera). (Figs. 2A; 3A). C-p1–5 scale was used to represent the guilds, with c-p2 (10 genera) nematodes typically predominating (found in 54% of samples; see Figs. 2B and 3A). For the bacterivores group, Cephalobus was the most predominant (PV= 91.1; MPD= 101.3) found in 86.1% of soil samples. This was followed by Rhabditidae (PV= 79.9; MPD= 92.2) found occurring in 75% of the soil samples. The third most predominant Bacterivore was Eucephalobus (PV= 42.2; MPD= 67 %) recorded from 55.6% of the samples. Pseudacrobeles and Prsimatolaimus were the 2 least predominant Bacterivores from the kola field samples having PVs of 10.4 and 7.3 and found in 30.6% and 16.7% of the samples respectively. In terms of the fungivores, Aphelenchus was the most predominant (PV= 31.1; MPD= 38.4) found in 61.1% of samples. Regarding the omnivores from this study, Aporcelaimidae was the most predominant (PV= 46.8; MPD= 45.8) found occurring in 83.3% of the soil samples (Table 1). The second most predominant was Aporcelinus (PV= 25.4; MPD= 47.9) found present in 33.3 % of soil samples from the kola tree fields. The least predominant omnivores were Sectonema (PV= 10.5; MPD= 25.8) recovered from 16.7% of the samples. Result of plant parasitic nematodes (PPNs) from this study revealed that Helicotylenchus (PV= 63.2; MPD= 68.7) was the most predominant found occurring in 83.3% of soil samples while second most predominant PPN was Tylenchulus (PV= 17.4; MPD= 21.3) found occurring in 55.6% of the soil samples collected. Meloidogyne was the least predominant PPN recovered from the kola tree fields (PV= 5.3; MPD= 7.6) found in 47.2 % of the samples. Mononchus was the most predominant predatory nematode (PV= 45.8; MPD= 59.1) recovered from 61.1 % of the samples while the 2 least predominant predatory nematodes from the kola tree fields were Parahandrochus and Mylonchulus with PVs of 26.5 and 20.9 respectively (Table 1).

The c-p triangle and metabolic footprints were used to establish the ecological indices for the faunal analysis of nematode data (Fig. 4). Maturity indices ranged between 2.51 – 2.97 (Mean = 2.76) while mean Channel index (CI), Enrichment index (EI) and Structural index (SI) were 21.49, 45.33 and 77.98 respectively which indicates that most of the fields were nearly stable soil conditions without enrichment. Plant parasitic index/maturity index (PPI/MI) ratio ranged from 1.02 to 1.32 (mean =1.13) depicting a moderate proportion of PPNs to other nematode groups present. No significant difference ($P \leq 0.05$) was observed across all indices of ecosystem measured except in terms of the mean plant parasitic index and PPI/MI ratios (Table 2). Result of the food web analysis as depicted by the metabolic footprints showed that 25% of samples were plotted in quadrat B, representing maturing and enriched soil conditions while the rest 75 % lotted in quadrat C which was described as having stable and fertile soil conditions (Fig. 3A and B).

It has been determined that nematodes are excellent markers of the health of soil. The division of nematodes into trophic groups, feeding groups, food web/faunal analysis, and metabolic footprints of nematode groups have all significantly improved this technique (Yeates et al. 1993; Bongers and Bongers 1998; Ferris, 2010). Several studies have been conducted worldwide to provide insights into nematode community structures in different natural and agro-ecological systems (Schorpp and Schrader 2017; Tian et al. 2020).

Table 1. Calculated mean population densities (MPD), frequencies of occurrence (FO%), prominence values of individual nematode taxa recovered from kola tree fields in Nigeria.

Feeding group/Genus	***Family***	***Guilds***	***FO%***	***MPD***	***PV***
Bacterivores					
Cephalobus	*Cephalobidae*	Ba-2	86.1	101.3	91.1
Rhabditis	*Rhabditidae*	Ba-1	75.0	92.2	79.9
Eucephalobus	*Cephalobidae*	Ba-2	55.6	67.0	42.2
Acrobeles	*Cephalobidae*	Ba-2	66.7	45.4	35.1
Monhystera	*Monhysteridae*	Ba-2	44.4	44.1	29.4
Plectus	*Plectidae*	Ba-2	38.9	21.8	14.3
Pseudacrobeles	*Cephalobidae*	Ba-2	30.6	19.5	10.4
Prismatolaimus	*Prismatolaimidae*	Ba-3	16.7	19.2	7.3
Fungivores					
Aphelenchidae	*Aphelenchidae*	Fu-2	61.1	38.4	31.1
Paraphelenchus	*Aphelenchidae*	Fu-2	13.9	22.0	9.2
Omnivores					
Aporcelaimidae	*Aporcelaimidae*	Om-5	83.3	45.8	46.8
Aporcelinus	*Aporcelaimidae*	Om-5	33.3	47.9	25.4
Discolaimus	*Discolaimidae*	Om-4	36.1	35.4	21.3
Achromadorus	*Achromadoridae*	Om-3	52.8	28.7	20.8
Laimydorus	*Dorylaimidae*	Om-4	27.8	21.5	11.3
Sectonema	*Aporcelaimidae*	Om-5	16.7	25.8	10.5
Herbivores					
Helicotylenchus	*Hoplolaimidae*	PL-3	83.3	68.7	63.2
Paratylenchus	*Tylenchidae*	PL-3	55.6	21.3	17.4
Pratylenchus	*Pratylenchidae*	PL-3	61.1	16.4	13.1
Hoplolaimus	*Hoplolaimidae*	PL-3	33.3	21.3	12.3
Criconema	*Criconematdae*	PL-3	58.3	13.8	10.5
Xiphinema	*Longidoridae*	PL-5	75.0	10.6	9.1
Ditylenchus	*Anguinidae*	PL-2	69.4	9.8	8.2
Meloidogyne	*Meloidogynidae*	PL-3	47.2	7.6	5.3
Predators					
Mononchus	*Mononchidae*	Pr-4	61.1	59.1	45.8
Parahadronchus	*Mononchidae*	Pr-4	50.0	38.9	26.5
Mylonchulus	*Mylonchulidae*	Pr-4	47.2	31.5	20.9

Ba= Bacterivores; Fu= Fungivores; Om= Omnivores; PL= Plant feeders (PPN); Pr= Predators

Table 2. Indices of ecosystem functions expressed by nematode taxa recovered from kola tree fields in Nigeria

Index name	Site A	Site B	Site C	Site D	ANOVA, p	Mean	SD
Maturity Index (MI)	2.76	2.97	2.8	2.51	0.32	2.76	0.19
Maturity Index 2-5 (MI2-5)	3.21	3.17	3.01	2.81	0.16	3.05	0.18
Sigma Maturity Index (SMI)	2.84	3.04	2.85	2.6	0.22	2.83	0.18
Plant Parasitic Index (PPI)	3.09	3.02	3.02	3.32	0.01	3.11	0.14
PPI/MI	1.12	1.02	1.08	1.32	0.03	1.13	0.75
Channel Index (CI)	19.92	20.37	36.6	9.07	0.37	21.49	11.35
Basal Index (BI)	12.66	17.87	19.07	19.97	0.43	17.39	3.27
Enrichment Index (EI)	65.1	29.97	40.42	45.82	0.10	45.33	14.73
Structure Index (SI)	82.32	80.16	77.87	71.57	0.21	77.98	4.64
Composite footprint (CF)	506.46	346.4	444.78	435.17	0.19	433.20	65.93
Enrichment footprint (EF)	173.7	57	95.93	104.83	0.12	107.87	48.56
Structure footprint (SF)	279.17	201.21	185.8	235.21	0.41	225.35	41.40
Total biomass (mg)	2.44	1.83	2.16	1.95	0.43	2.10	0.27

In Nigeria and west Africa, the very few information available about nematode community structure came from arable crops production systems. Previous studies from Nigeria which addressed free-living nematode community structures were those from Eche et al. (2013) who identified 43 genera of free-living nematodes from citrus, maize and yam fields of north central region of Nigeria. Also, Alabi et al. (2017) reported 4 genera from yam fields in southwest Nigeria. Therefore, to the best of our knowledge, no known study exists in respect of any forest ecological systems in Nigeria. Our present study is the first in this regard, using kola tree forests as a template for using nematode community structures in other forest ecosystem diversity and functioning studies in West Africa

In the current study, a total of 8 genera of plant parasitic nematodes with Helicotylenchus and Paratylenchus being the most predominant. Although, these two nematodes are highly destructive in many ornamental crops worldwide (Bell and Watson 2001: Davis et al. 2004; Howland and Quintanilla 2023), their predominance in kola tree fields is interesting being contrary to previous reports of PPNs abundance across southwest Nigeria where root-knots (Meloidogyne spp.) are known to be the most predominant PPNs (Bello et al. 2020). Therefore, build-up of high population of these two destructive nematodes must be discouraged since this might pose a threat to kola nut production in this region in the future.

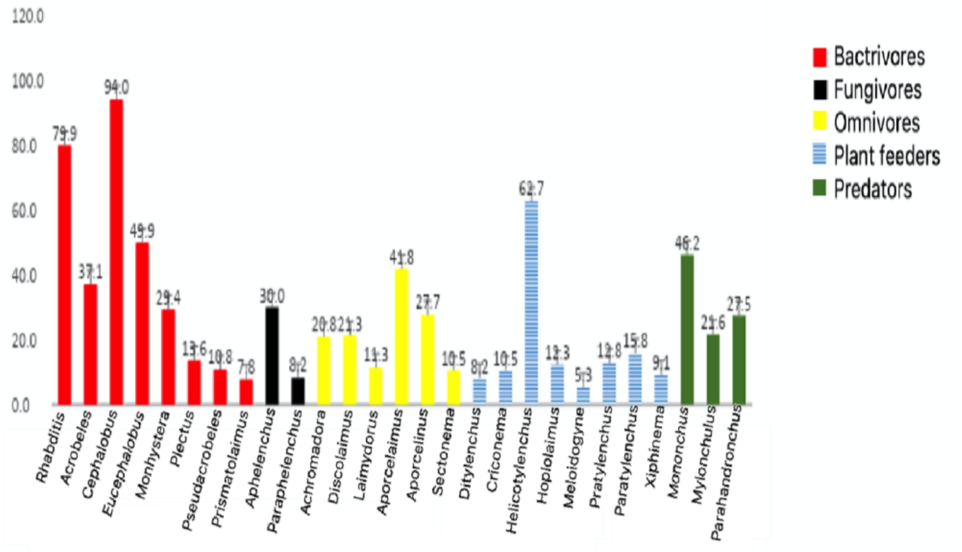

Figure 1: Mean prominent values of nematode taxa recovered from kola nut fields in Nigeria.

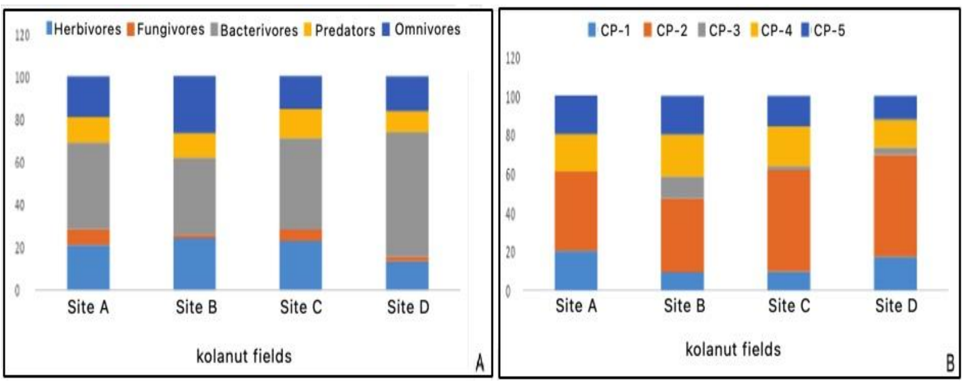

Figure. 2. A. Relative percentage of feeding type composition of nematode assemblages from kola fields in Nigeria. B. Relative percentage of colonizer-persister (C-P) structure of nematodes assemblages from kola fields in Nigeria

Bacterivores nematode group was the most predominant free-living nematodes from this current study which supports all earlier reports from Nigeria (Eche et al. 2013; Alabi et al. 2017). Generally, bacterivore nematodes have reproductive capability and short lifecycle which is a sign of enhanced soil conditions (Du Preez et al. 2022). Furthermore, it is also an established fact that community structures of soil organisms are largely dependent upon autotrophic inputs of plant as well as subsidiary inputs from other sources as depicted by enrichment and soil conservation practices (Ferris and Bongers 2006).

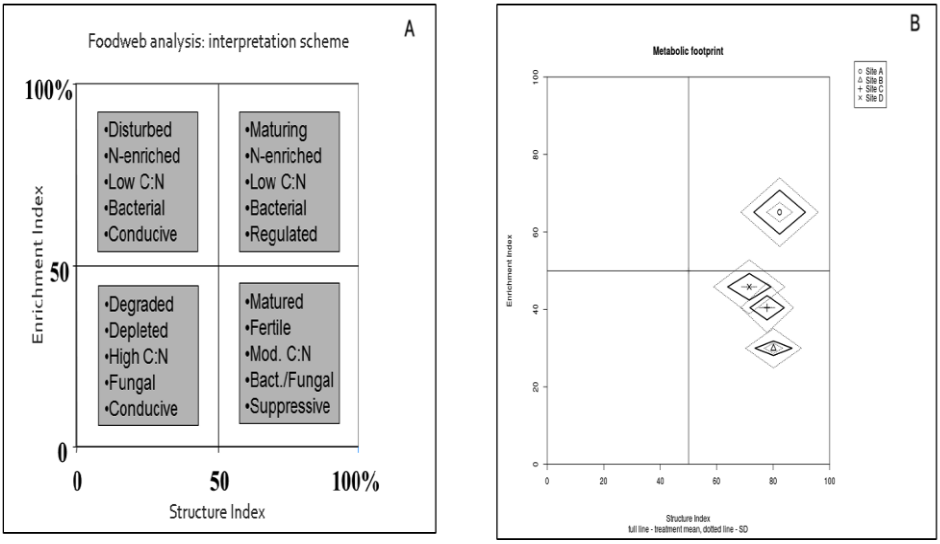

Figure 3. A. Food web analysis interpretation scheme. B. Metabolic footprints of nematodes assemblages from kola fields from Nigeria.

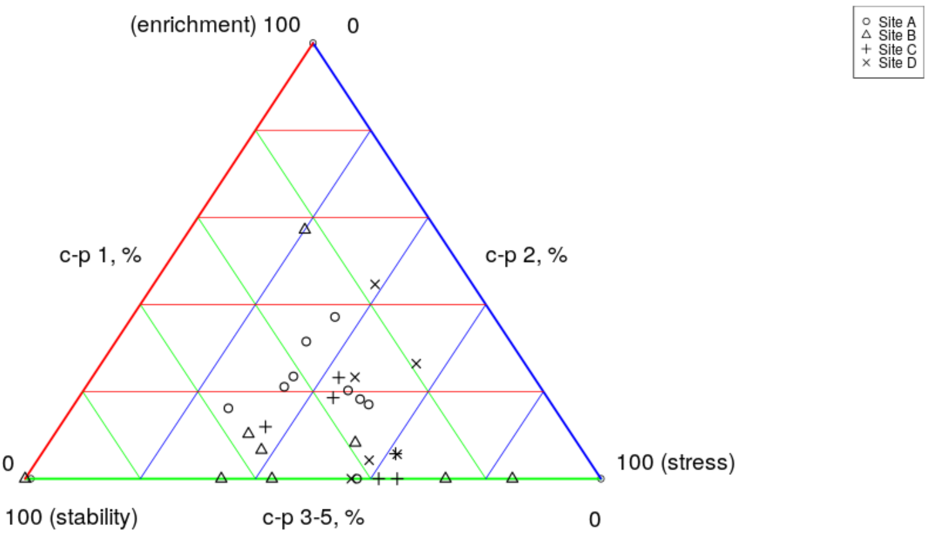

Figure 4. A c-p triangle of individual unweighted proportional representation of each c-p class represented in the nematode assemblage of kola fields in

The two most predominant free-living bacterivores nematodes from our current study: Cephalobus and Rhabditis, belong in the cp-2 and cp-1 groups respectively which is an indication of a mixture of both enriched and stressed soil conditions. Aporcelaimidae (cp-5) being the most predominant omnivores nematode recovered from this study confirms earlier reports of high abundance of the Aporcelaimidae from other cropping systems within south west Nigeria (Rashidifard et al. 2021; Bello et al. 2022b). Our findings further emphasize the importance of utilizing the concept of nematode c-p values in interpreting food web status of different soil habitats. The high mean maturity indices (MI) recorded from the fields is an indication of absence of N-fertilization or manuring since according to Bongers et al. (1997), MI values are known to decrease significantly as a result of manuring of any form.

Furthermore, Bongers et al. (1997) identified a correlation between PPI/MI ratio and soil disturbance. Therefore, we can say that the low PPI/MI ratio recorded from this current study is attributable to the low disturbance experienced due to the fact that no tillage activities was reported from any of the kola tree fields sampled in the last fifteen years. The proportionate contributions of the cp-3-5 nematodes to the cp-2-5 nematode groups are described by the structural index (SI) (Ferris et al. 2001). The high SI values recorded from our current study agrees with previous data from other forest ecosystems (Ferris et al. 2004; Cardosso et al. 2016). This also further buttresses the fact that EI, MI and SI values are important indices for measuring ecosystem functions using nematodes as indicators. Our findings further support the importance of utilizing the concept of nematode c-p values in interpreting food web status of different soil habitats. Majority (75 %), of the soil samples obtained from the kola tree fields plotted in quadrat C, was described as stable soil conditions, while the remaining 25% plotted in quadrat B represent maturing soil conditions. This implies that soils from most of the kola tree fields are in either a stable or maturing food web conditions which are characterized by low disturbance to moderate disturbance; also having a balanced bacterial mediated decomposition channels due to low C/N ratios. The results of our current study therefore agree with earlier reports which suggest a near balanced decomposition channels in most forest ecosystems that is devoid of disturbance (Waring and Running 2010).

Nematodes being the most abundant metazoan in the soil plays a crucial role in virtually all soil processes and has been identified as being an excellent indicator of soil conditions. The current study contributes to the existing information regarding the use of nematodes as soil health indicators and furthermore provides the first study of nematode assemblages in this case for kola tree fields in West and Sub-Saharan African region. Thus, comprehending that the dynamics of soil health is crucial to discerning how our ecosystem as a whole respond to problems with soil health.

CONCLUSION

Microbiome in soil consists of microbiota, macrofauna, mesofauna and microfauna, thus forming the complexity of food web. Flow of energy is supported in a properly arranged food web. This characterizes a perfect and healthy ecosystem that is primary to crop production. Food web state in soil is pivotal for forethought to achieve affordable management of cultivable lands. A representative of multiplex trophic zones in food web are nematodes. Hence projecting them as premium organisms in soil health evaluation. Helicotylenchus and Tylenchulus are the prominent pathogenic nematodes identified. More studies on the pathogenicity of the prominent plant parasitic nematodes on kola are needed in order to discourage their build up so as to avert impending problem in the future

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EVALUATING LANDSLIDE HAZARD IN THE BOUDINAR BASIN (MOROCCO) THROUGH SOIL PROPERTIES

SUMMARY

This research assesses landslide hazards in the Boudinar Basin (Morocco) through an evaluation of the area's soil properties and their interaction with the landslide processes. From exhaustive soil sampling and analysis, three distinct zones of landslide susceptibility were established: northern, central, and southern areas. The northern area, which is relatively flat, has a low carbonate content and a very high organic matter, contributing to low landslide risk. In contrast, the central area has a relatively moderate landslide risk due to the great number of samples with moderate organic matter and high carbonate that increases landslide hazards. Southern areas which have steep slopes are very prone to landslides as a result of great mass movement. These results show the predominant role of the topographic factor and the nature of soils in the landslide risks assessment and underline the need for advanced soil conservation and risk management measures in the Boudinar Basin.

Keywords: carbonate, organic matter, slope, soil

INTRODUCTION

The mountainous regions of northern Morocco are prone to significant landslide risks. This susceptibility is heightened by both geological factors, such as tectonic activity in the eastern areas like Al-Hoceima and Driouch (Taher and Mourabit, 2022; Taher *et al*., 2024), as well as climatic conditions, particularly notable precipitation levels in the western regions including Tanger, Tetouan, and Chefchaouen. These factors, combined with fragile lithology like shale, contribute to the prevalence of landslides in the Rif region. Data from field surveys conducted by the Moroccan Provincial Directorate of Practical Work reveal a history of landslides in the region. For instance, up until 1992, Chefchaouen province experienced 47 landslides, Tanger 17, Tetouan 19, and Al-Hoceima one. Recent data, such as that from 2004 to 2016, indicates a significant

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increase in landslide occurrences. For example, Al-Hoceima saw 151 landslides during this period (Byou, 2021). As a result, infrastructure such as roads (fig. 1), bridges, and communities are increasingly vulnerable to landslide hazards.

In recent years, progress in remote sensing and geographic information system (GIS) technologies has significantly enhanced the classification of various factors that trigger landslides, facilitating the creation of detailed landslide inventory maps (Gupta, Pal and Das, 2022; Ait Omar *et al.*, 2024). The utilization of GIS tools for generating, managing, and analyzing geographic data has become pervasive, particularly in forecasting potential landslide hazards (KC, Dangi and Hu, 2022). A range of GIS-based methodologies has been employed for landslide susceptibility mapping, including the Analytical Hierarchy Processes (AHP) (Afzal et al., 2022; Taher et al., 2025), the Frequency Ratio model (FR) (Sheng *et al*., 2022), and machine learning (ML) techniques (Trinh *et al*., 2022).

Figure 1 : The photos depict landslides threatening roads in the Boudinar basin

Many studies have demonstrated the influence of soil properties on landslide processes. For example, Louino et al. (2022) found in Lombardy, Italy, that coarse-textured soils are more prone to landslides compared to fine-textured soils. Similar results were reported by Cerri et al. (2020) in the Serra do Mar Mountain range in Brazil. The increased pore pressure due to water saturation also raises the risk of landslides, highlighting the importance of understanding the dynamic interaction between soil hydrology and landslide processes (Sidle et al. 2016; Bogaard et al. 2016). From these previous studies, we conclude that soil properties play a critical role in landslide dynamics.

In this context, the main objective of this study is to evaluate the impact of various soil properties on landslide processes. Specifically, we focus on key factors such as organic matter content, and carbonate levels. Understanding the role of these properties is essential for identifying areas prone to landslides and improving risk assessment and mitigation strategies. Through this study, we aim to provide valuable insights into the dynamic interactions between soil characteristics and landslide susceptibility, contributing to a more comprehensive understanding of the underlying mechanisms driving these natural hazards

MATERIALS AND METHODS

Study area

The Boudinar Basin, located in the northeastern region of Morocco (Fig. 2), is situated within the administrative boundaries of the Driouch province and encompasses several municipalities, including Trougout, Boudinar etc.

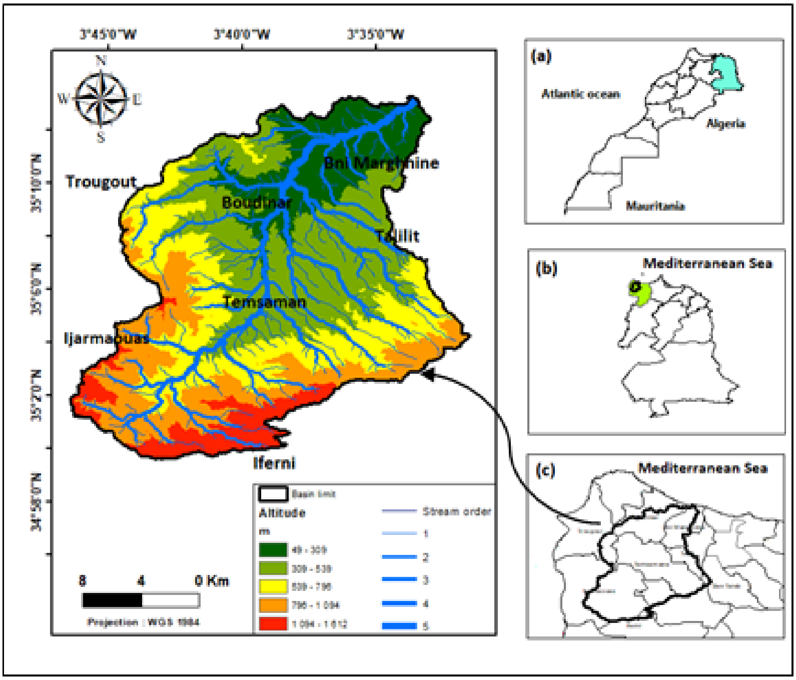

Figure 2 : The Geographical and administrative location of the Boudinar Basin (Taher et al., 2023)

Through the application of Geographic Information System (GIS) techniques, the basin has been precisely delineated, covering an expansive area of 350 square kilometers. Extending from latitude 35.22 to 34.99 north and longitude 3.52 to 3.77 west, its geographical expanse is substantial (Taher et al., 2023). The primary watercourse within the basin is the Oued Amakran, which traverses approximately 40 kilometers before reaching its terminus at the Mediterranean Sea. The altitude profile within the study area presents a diverse topography, with elevations ranging from 49 to 1612 meters above sea level. This variation in altitude contributes to the dynamic landscape of the Boudinar Basin, shaping its ecological and geological features. Additionally, the slopes within the basin exhibit a wide spectrum, ranging from gentle inclines of 0 degrees to steep gradients of up to 63 degrees.

This variability in slope inclination further adds to the complexity of the basin’s terrain, influencing factors such as soil erosion, land use suitability, and hydrological processes (Taher et al., 2023).

Soil sampling

Ten soil samples were collected from the Boudinar basin during field mission, with their coordinates noted using GPS (fig. 3). In the geology laboratory at the Faculty of Science and Technology in Al Hoceima, five soil properties of Boudinar basin soil were determined, soil pH was assessed using a palliasse pH meter in a soil-water suspension (fig. 4D). Additionally, bound water, organic matter, and carbonate contents were determined through loss on ignition using high accuracy balance and drying furnaces (fig. 4A&C). Magnetic susceptibility was measured directly using a portable SM30 susceptibility meter with a sensitivity of 10-3 SI and an operating frequency of 8 kHz (fig. 4B) (Dakiri et al., 2021; Tawfik et al., 2024).

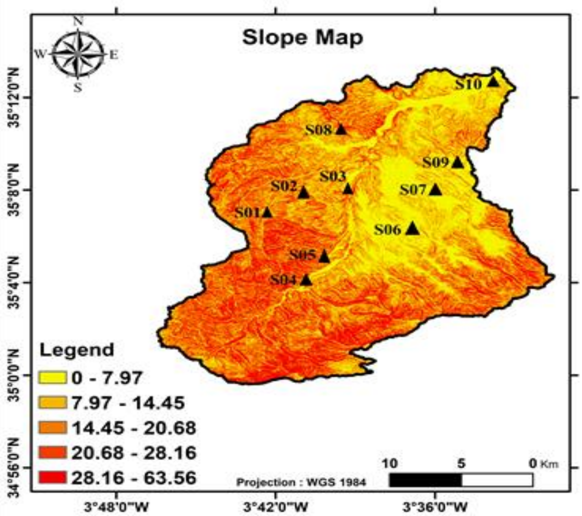

Figure 3: Soil sampling locations in the Boudinar basin

Figure 4: Laboratory equipment employed for the examination of the soil properties attributes of Boudinar soil samples includes: A: analytical balance B: susceptibility meter, C: drying furnaces, D: pH meter

Multi-Criteria Decision Analysis (MCDA)

A comprehensive landslides susceptibility map was developed by integrating nine key parameters. These parameters underwent classification through a weighted overlay analysis and MCDA technique. The rainfall distribution map was meticulously crafted using the inverse distance weighted (IDW) method, drawing upon data from the Power Larc Project, supported by NASA, spanning the years 2010 to 2021. Faults and lithology were mapped digitally, referencing the Boudinar geological map at a scale of 1/50000, sourced from the geological survey of Morocco. Road networks were digitally mapped using data from Google Earth. Furthermore, a digital elevation model (DEM) sourced from the Earth Data (NASA) website was utilized to generate topographic layers such as aspect, slope, and elevation. Finally, NDVI maps were produced using Landsat 9 OLI data acquired from the United States Geological Survey (USGS).

RESULTS AND DISCUSSION

Results

Soil properties of the Boudinar basin

Table 1 offers information on many soil properties, including pH, Bound Water (BW%), Organic Matter (OM%), Carbonate ($CaCO_3$%), and Magnetic Susceptibility (X), therefore illuminating the features and behaviour of the soil.. The pH values range from 8.014 to 8.572, indicating consistently alkaline conditions across all samples. Bound Water (BW%) varies between 1.10% and 4.38%, reflecting the amount of water tightly bound to soil particles, with higher values suggesting greater water retention capacity.

Table 1 : The pedological characteristics of soil samples

Soil sample N°	Longitude	Latitude	pH	BW%	OM%	$CaCO_3$	X(10^{-6}SI)
S01	3.71301	35.12664	8.014	1.85	1.56	0.16	4000
S02	3.68038	35.13802	8.513	1.57	1.88	2.90	92
S03	3.63966	35.10393	8.330	3.67	2.91	3.14	2625
S04	3.68120	35.06073	8.389	2.88	1.94	0.97	1670
S05	3.66928	35.07542	8.572	1.77	1.29	1.41	1235
S06	3.61061	35.11447	8.528	1.86	1.99	2.49	2490
S07	3.60037	35.13375	8.527	2.03	1.84	3.78	782
S08	3.65865	35.18137	8.542	1.10	1.25	1.63	509
S09	3.58561	35.15503	8.350	2.37	2.09	5.68	2460
S10	3.56610	35.21595	8.324	4.38	2.69	1.99	42

BW: Bound Water; OM: Organic matter; $CaCO_3$: Carbonate; X: magnetic susceptibility

Organic Matter (OM%) ranges from 1.29% to 2.91%, which is vital for soil fertility and nutrient retention. Carbonate content ($CaCO_3$%) fluctuates from 0.16% to 5.68%, influencing soil structure and pH levels. Lastly, Magnetic Susceptibility (X) spans from 42 to 4000, with higher values potentially indicating the presence of magnetic minerals such as magnetite or iron oxides, which can affect both soil properties and fertility.

Landslide Susceptibility Map

The Landslide Susceptibility Map of the Boudinar basin has been categorized into three classes: low, moderate, and high, as depicted in Figure 5. The high susceptibility category, encompassing 88 km2 of the study area, is predominantly concentrated in the southern and southeastern regions. The analysis suggests that slope, lithology, altitude, and NDVI are the most influential factors contributing to landslide susceptibility. In contrast, the moderate susceptibility category, covering 242 km2 of the total area, is predominantly situated in the central part of the Boudinar basin. The low susceptibility class, accounting for 18 km2, is primarily located in the northern region.

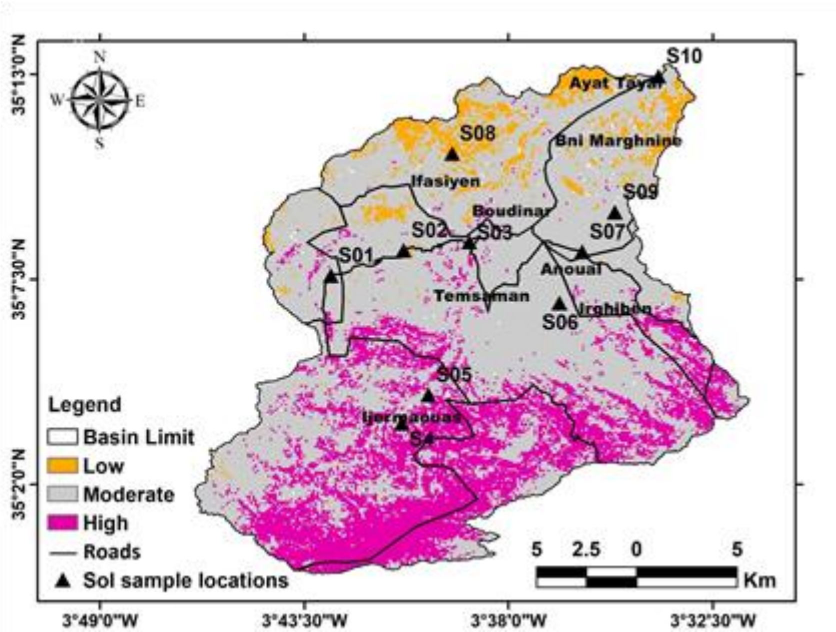

Figure 5: Landslide susceptibility map of the Boudinar basin

Discussion

The map (figure 5) shows distinct landslide susceptibility classes (low, moderate, and high) across the Boudinar basin. These classes correlate with the spatial distribution of soil properties, and slope map (figure 3):

The northern part of the basin is predominantly categorized as having low landslide susceptibility. The soil properties of this area are characterized by low carbonate content, with sample S10 showing 1.99% and sample S08 at 1.63%. This low carbonate content could lead to reduced soil cohesion when saturated (Kemper, Rosenau, and Dexter, 1987). Additionally, sample S10 exhibits high organic matter at 2.69%, which helps stabilize soils by enhancing root binding and reducing erosion (Tisdall and Oades, 1982). However, the flat terrain and stable geological conditions further minimize landslide risk (figure3).

The central region is predominantly characterized by moderate landslide susceptibility. Soil properties in this area show that most samples contain moderate organic matter and high carbonate levels, both of which contribute to an increased landslide risk. Eskene's study highlights that variations in near-surface soil carbonate profiles are closely linked to soil erosion and deposition, with its concentrations typically rising in erosional zones. This suggests that high carbonate content is associated with soil displacement and surface instability, factors that exacerbate erosion and landslide risks (Erskine *et al*., 2017). For instance, at site S03, high bound water content (3.67%) increases pore-water pressure, which can destabilize slopes during heavy rainfall. The combination of moderate slopes, moderate organic matter, high carbonate levels, and moisture-retaining soils in this region contributes to its moderate susceptibility to landslides.

The southern part of the basin is predominantly characterized by high landslide susceptibility. The soil characteristics in this section of the study area do not exhibit specific patterns in carbonate content or organic matter. The primary factor contributing to landslide risk here is topography, as depicted in Figure 3, where the area is defined by very steep slopes. Vanacker *et al*. (2019) emphasize that topography plays a crucial role in controlling soil movement and the redistribution of pedogenic material, with steep slopes showing clear spatial differences in weathering and increased material fluxes.

In light of these results, organic matter plays a critical role in the initial stages of soil formation on landslides, positively affecting microbial biomass and enzyme activities essential for soil recovery and stability (E. Błońska *et al*., 2018). Moreover, its addition to soil can significantly reduce the duration and volume of landslides, even under varying rainfall intensities, suggesting that organic matter enhances soil cohesion and stability, thereby mitigating landslide impacts (Rofiq et al., 2022). On the other hand, carbonate content in soil plays a significant role in landslide dynamics by affecting soil stability and chemical properties. The dissolution of carbonates can weaken soil structure, making it more susceptible to landslides, especially in areas with steep slopes and heavy rainfall (Ying *et al*., 2020; Forte *et al*., 2019; Cheng *et al*., 2016).

CONCLUSION

The study of the spatial distribution of soil properties across the Boudinar Basin has shown a moderate correlation with landslide susceptibility. However, other factors also contribute to landslides. Therefore, it is essential to correlate these findings with additional factors, such as geological and anthropogenic influences.

In conclusion, the landslide susceptibility in the Boudinar Basin varies across its regions due to differences in soil properties and topography. The northern region, with its low carbonate content, high organic matter, and flat terrain, has a low susceptibility to landslides. In contrast, the central region, characterized by moderate slopes, moderate organic matter, high carbonate levels, and moisture-retaining soils, exhibits a moderate landslide risk. The southern region, marked by very steep slopes, experiences the highest landslide susceptibility, with topography being the dominant factor influencing landslide risk. Overall, the study highlights the significant role of both soil properties and topography in determining landslide susceptibility in the basin.

As a perspective, we recommend that future research investigate soil properties using multiple deep soil samples across the study area to better evaluate landslide risks. Understanding these relationships can aid in effective land management and mitigation strategies to reduce landslide risk in vulnerable areas.

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GEO-SPATIAL LAND-USE CHANGE DYNAMIC ASSESSMENT IN CONSECUTIVE PERIODS UNDER THE AGRICULTURAL EXPANSION DRIVING FORCE

SUMMARY

Assessing land use change rate and extent is crucial in global environmental change and sustainable development. The study employed Landsat satellite imagery from MSS, TM, ETM+, and OLI sensors to identify land use changes between 1988 and 2018. These images underwent SVM classification to categorize land use. Dynamic land use change was assessed via single and integrated indices. The fastest change, at 1.40%, occurred in tree lands between 2010 and 2018, while the slowest changes were seen between 1988-2000 and 2000-2010. Water bodies showed the highest intensity of change during 1988-2000, at +0.37%. The dry farming demonstrated the least changes in all three periods. The pasture underwent significant changes in the periods of 1988-2000 and 2000-2010, registering high intensities at 0.288% and 0.31%, respectively. The integrated dynamic land use change was 0.14% during 1988-2000 and 0.06% in 2010-2018, totaling 0.245% over the 30-year study period. Agricultural land use expansion has been a dominant force driving land cover changes in the study watershed. The conversion of natural areas into agricultural lands highlights the need for sustainable land management practices to mitigate environmental impacts while meeting agricultural demands. The study emphasizes the significance of land use dynamic degree as a valuable tool for managers and helps identify driving forces behind land use change intensity across different periods, and predict future land use changes.

Keywords: Agricultural expansion, Land use change, Socioeconomic drivers, Dynamic degree, Sustainable land management

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INTRODUCTION

Land use and land cover are key factors in global environmental change and sustainability (Uralovich *et al.*, 2023). The interactions related to land use change include the response to climate change, effects on ecosystem structure and function, and water and energy balance (Bello *et al*., 2018; Adeyeri *et al*., 2023). The Earth's surface has undergone changes and modifications throughout its history, occurring at different spatio-temporal scales, both in short and long-term periods, which may be reversible or permanent (Bishop, 2007; Shen *et al*., 2023). Land use and land cover change refer to the conversion of land use types resulting from complex interactions between humans and the physical environment (Liping *et al*., 2018). Key consequences of LULC change include intensified soil erosion due to loss of protective vegetation, increased flood risk from reduced soil permeability and higher runoff, biodiversity loss, and declining water quality (Spalevic *et al*., 2017; Spalevic *et al*., 2020). Land use change is the central driver and the most dynamic phenomenon between human and ecological systems. Along with the nature of the environment, changes in land use and land cover patterns must be taken into account considering complex factors related to technology, demand, and social relationships (Pande *et al*., 2018; Talebi Khiavi *et al*., 2022). Detecting changes in land use involves determining the process, extent, and rate of observed changes during different periods (Iqbal and Iqbal, 2018; Shekar, and Mathew 2023). Change detection is the process of identifying differences in the state of a feature or phenomenon by observing it at different times. Remote sensing combined with Geographic Information System (GIS) techniques can improve the efficiency of identifying land cover changes (Zhang *et al*., 2009; Chaikaew, 2019). Multi-temporal remote sensing (RS) based on change detection analysis has been repeatedly used in different aspects of LULC change detection and environmental resources assessment (Juliev *et al*., 2019; Abdolalizadeh *et al*., 2019). Remote sensing data covers large geographic extents, and this high spatial resolution data provides valuable information regarding the process, location, rate, trend direction, pattern, and magnitude of LULC changes. Meanwhile, GIS is useful for mapping and analyzing the patterns captured by the remotely sensed data. The use of remote sensing and GIS technologies has added a new dimension to the interpretation and understanding of LULC dynamics. LULC change and its effects on the world's environment are among the main research topics (Zhang *et al*., 2011). Many studies have investigated specific facets of land use and land cover (LULC) change, yet a comprehensive understanding of the dynamics driving these changes remains underexplored and demands greater focus (Xinliang and Jiyuan, 2003; Xinchang *et al*., 2004).

The dynamic degree of land use change serves as an effective tool for evaluating the rate, intensity, and extent of land use transitions over a specific period (Liu *et al*., 2017). Previous research primarily concentrated on calculating land use dynamic indices, analyzing transfer matrices, and identifying the driving forces behind landscape changes. Chunxiao *et al*. (2008) analyzed land use changes in Jilin province (1988–2000) using remote sensing and GIS, finding a shift from grassland, woodland, and bare land to cultivated areas, with grassland showing the highest dynamics due to human activities. Sun *et al*. (2013) assessed Genhe City (2000–2010) and reported forest conversion to built-up areas as dominant, with land use change intensity declining over time. Zhang *et al*. (2014) studied hydrological impacts of LULC changes in sub-watersheds, noting dynamic indices of 2.29%–7.99% for forest, brush, and built-up areas. Liu *et al*. (2018) used a transfer matrix to analyze the Huaihe basin (1985–2014), reporting high dynamics in artificial water bodies and construction land. Talebi Khiavi and Mostafazadeh (2021) found residential areas had the highest change (7.97%) in their analysis (1984–2016), with forest cover declining (−1.85%). Wang *et al*. (2022) predicted urban expansion in Wuhan (1990–2015), reducing arable and woodland areas. Wang and Wang (2022) projected urban growth and forest decline in Wuhan by 2030, emphasizing sustainable land use. Hou *et al*. (2022) reported cropland growth in the Tarim River Basin (1992–2020), projecting grassland expansion and barren land decline. Hussain *et al*. (2024) highlighted urban growth and vegetation loss in Multan, predicting increased urban heat stress by 2050. Determining rates and magnitudes of change within specific land use types, can shedding light on the differential impacts of human activities on different landscapes and facilitating targeted interventions for sustainable land management across diverse geographical regions and temporal scales (Mohammed *et al*., 2024). The literature review indicates that the dynamics of land use and cover change are influenced by various driving forces (Valverde, 2023). Driving forces of land use change vary across regions (Assede *et al*., 2023), and satellite imagery serves as a valuable tool for understanding the environmental impacts of human activities and predicting future LULC changes (Twisa and Buchroithner, 2019; Vivekananda, 2021).

This study examines critical land-use dynamics in arid watersheds, where agricultural expansion challenges ecosystem preservation. Using the Kozehtopraghi watershed as a representative case, our 30-year analysis reveals how socioeconomic factors drive changes in vulnerable agro-pastoral systems. The combination of transition matrices and dynamic degree metrics provides a replicable method for quantifying change rates. The study area, characterized by diverse land uses influenced by economic activities, exploitation types, population factors, topography, and climate, was selected to assess land use change dynamics. The aim of this study was to assess land use changes and calculate the dynamics of land use change in the Kozehtopraghi watershed in Ardabil province from 1988 to 2018. Additionally, the extent, rate, and intensity of land use change in the study area were analyzed.

MATERIAL AND METHODS

2.1. Study area

The Kozehtopraghi watershed is located between latitudes 38˚ 07' 28" and longitude 48˚ 28' 10" geographic location. The GhoriChai river is the main river of the study area, draining an area of approximately 812.5 km². The Kozehtopraghi watershed is located in the south of Ardabil province, with lower and higher elevations of 1384 m and 2485 m above mean sea level, respectively (Alaei *et al*., 2022). Based on 40 years of recorded data, the mean rainfall and temperature are 300 mm and 6.95˚C, respectively. The residential region in this watershed consists of 65 villages, including Ghaleea Jogh, Khan Gheshlaghi villages, and Koraeim city (see Figure 1).

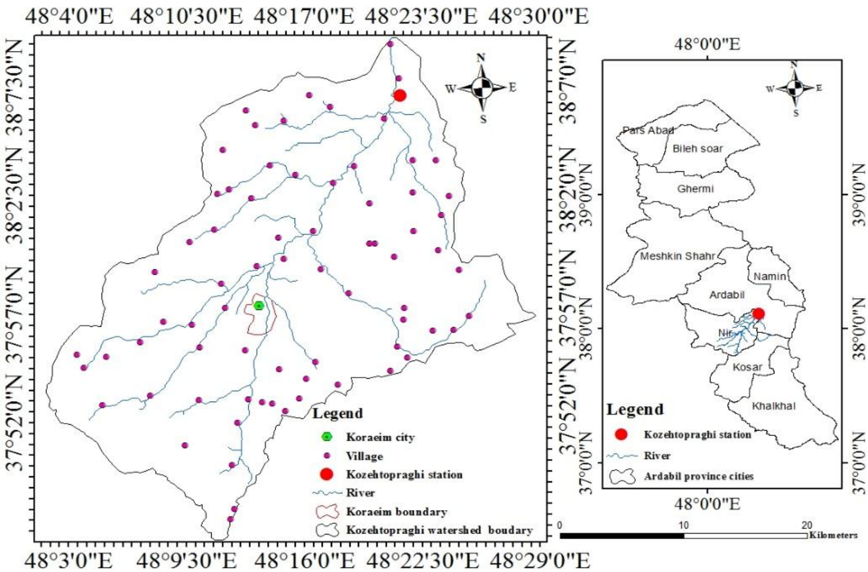

Figure 1. Location of the Kozehtopraghi Watershed in Ardabil province

Understanding the spatial pattern and location of different land covers and human activities in different parts of the watershed can aid in landscape management. The land use map is one of the sources of information that shows the results of various human activities at the watershed scale (Thenkabail, 2015). In this study, four Landsat satellite images from 1988, 2000, 2010, and 2018 were downloaded from the United States Geological Survey (USGS) official website (earthexplorer.usgs.gov), utilizing Landsat MSS, TM, ETM+, and OLI sensors to analyze 30 years of land use change (Rushema *et al*., 202). The Digital Elevation Model (DEM) of the study area was obtained from the PALOSAR sensor, projected, and used to prepare a slope map for the study area. The slope map was then layer stacked with the multi-spectral bands of each image for land use classification. Details of the satellite images used in the current research has been presented in Table 1.

Table 1. Detailed information of the satellite data used in this research

Sensor name	**Acquisition date**	**Spatial resolution**	**Spectral Bands**
MSS	198-07-30	60	Multispectral
ETM+	2000-06-05	Multispectral 30m Pan 15m	Multi spectral and Panchromatic
TM	2010-07-11	30 m	Multi spectral
OLI	2018-07-01	Multispectral 30m Pan 15m	Multi spectral and Panchromatic

In this study, the first step was to prepare the preprocessed land use map image in the ENVI 5.3 software environment. The steps of the present research are shown in Figure 2.

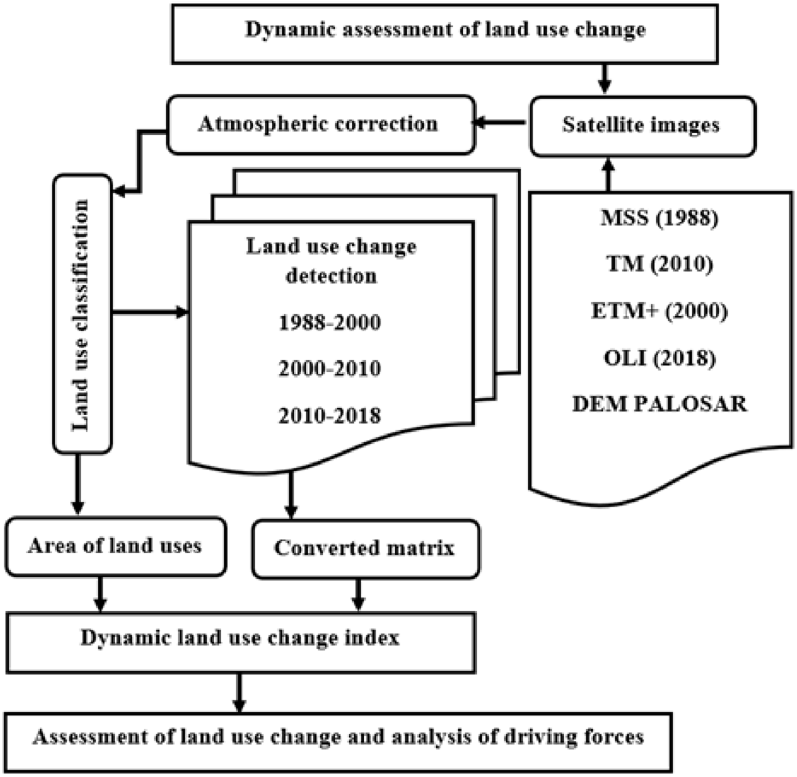

Figure 2. The methodology of assessing land use change dynamic

2.2. Methodology

2.2.1. Image Preprocessing

Satellite image preprocessing is usually done to improve land use and includes radiometric and geometric correction as well as image calculations. The purpose of atmospheric correction is to convert the high-level radiation received by the sensor to the radiation output of the earth. In this study, the FLAASH technique in ENVI 5.3 software was used for atmospheric correction, which utilizes image metadata. Since Landsat images are corrected to users, geometric correction was not performed on satellite images in this study. However, the geometric accuracy of the image was examined using several control points, which was found to be acceptable with an error of less than 15 meters.

2.2.2. Land sue classification

The decision on different spectral classes and their relationship with useful data classes is determined by the user and the purpose of the analysis. In this study, six classes (dry farming, irrigated agriculture, water-body, built-up, pasture, and tree land) were determined for the 2000, 2010, and 2018 images, and five classes (dry farming, irrigated agriculture, tree land, water-body, and pasture) were selected for the 1988 image. For each class, 250 training points were selected to ensure that all spectral classes corresponding to each land use category were adequately represented during the training stage. Additionally, the supervised classification method using Support Vector Machine (SVM) was applied to classify the land use in the satellite images. To assess the accuracy of the classified maps, 200 samples from each land use class were randomly selected (Zhang *et al*., 2011). After the image classification, an accuracy assessment was conducted on the thematic information (Shah *et al*., 2017). The accuracy assessment of the classification was carried out using ENVI 5.3 software.

2.2.3. Support Vector Machine algorithm

In this study, the classification was carried out using the non-parametric SVM classifier, with two hyperplanes selected. In SVM, it is not only important to maximize the distance between the two given land use classes, but also to ensure that no points fall between different land use classes. The aim was to determine the class into which new data points fall (Sibaruddin *et al*., 2018; Kranjčić *et al*., 2019).

d) Accuracy assessment of land use classification

Evaluating and understanding accuracy are essential prerequisites for using any information derived from thematic maps (Zhang *et al*., 2011). To evaluate the accuracy of classification results, training samples that are correctly classified are used, which is crucial for using and interpreting the results (Dehghani *et al*., 2023; Dehromi and Amiri, 2023). The accuracy assessment criteria include the overall accuracy (Equation 1) and Kappa coefficient (Equation 2).

$$\mathbf{OA = \frac{1}{N}\Sigma_{P_{ii}}} \tag{1}$$

Where, ΣPii represents the sum of the main diagonal elements. OA refers to overall accuracy, and N represents the number of testing pixels (Marin *et al*., 2021; Khavarian Nehzak *et al*., 2022).

$$\mathbf{Kappa = \frac{P_o - P_C}{1 - P_C} \times 100} \tag{2}$$

Where, P_O is observation accuracy, P_C is perspective harmony (Wang *et al*., 2012).

2.2.4. Land use change detection

Remotely sensed data is useful for monitoring land use changes in change detection analysis (Akyürek *et al*., 2018). Land use change detection has become a crucial aspect of studying areas for conservation and restoration purposes. With advancements in the field, this area of research has garnered significant attention from many researchers (Shah *et al*., 2017). Change detection analysis describes and quantifies differences between images of the same area at different times. The classified image of the four dates can be used to calculate the area of different land covers and observe changes that are taking place over time. The entire watershed was divided into six major land use classes, and LULC maps were generated based on the adopted classification. A change map was created from two images from different time periods, signifying areas with increases or decreases in pixel values above a specified threshold. The classified images were then used to produce a change matrix in ENVI 5.3 software (Shah *et al*., 2017).

2.2.5. Land use dynamic change assessment

The changes in land use within a given time period can be represented by a single land use dynamic degree, with its formula shown in Equation 3 (Khavarian Nehzak *et al*., 2022).

$$K = \frac{U_b - U_a}{U_a} \times \frac{1}{T} 100\%$$

Where, U_a and U_b represent the area of a certain land use type at the beginning and end of the research, where land use change in the research area is a result of changes in different land use types. Integrated dynamic land use change represents the overall changes of all land categories in the study period, with its formula as follows (Khavarian Nehzak *et al*., 2022):

$$LC = \left[\frac{\sum_{i=1}^{n} \Delta LU_{I-J}}{2\sum_{i=1}^{n} LU_I}\right] \times \frac{1}{T} \times 100\% \quad (4)$$

Where LU represents the area of a specific land use type at the beginning of the research, and, ΔLU_{I-J} represents the change in the area of that land use type during a specific time period T, where T is the year (Sun *et al*., 2013).

RESULTS AND DISCUSSION

Table 2 shows the overall accuracy and Kappa coefficient for the land use classifications of 1988, 2000, 2010, and 2018.

. **Table 2.** Accuracy assessment of land use classification for 1988, 2000, 2010, and 2018

Year	1988	2000	2010	2018
Overall accuracy	90	96	95.8	97.5
Kappa coefficient	0.86	0.95	0.94	0.96

Based on the land use classification results, the extent of different land uses in the Kozehtopraghi watershed for 1988, 2000, 2010, and 2018 was extracted to calculate the single dynamic land use change equation, with the results presented in Figure 3.

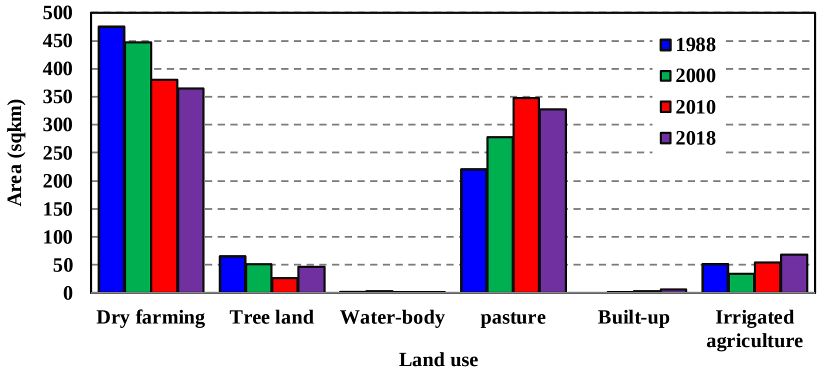

Figure 3. Area of land uses in 1988, 2000, 2010, 2018 in the study area

The land use conversion matrix of the Kozehtopraghi watershed for the four periods 1988, 2000, 2010, and 2018 was obtained using the classified images. Additionally, the land use change map of different periods have been presented in Figures 4, 5, and 6. Moreover, the numerical data of LULC change derived from land use conversion matrix are presented in Tables 3 to 6.

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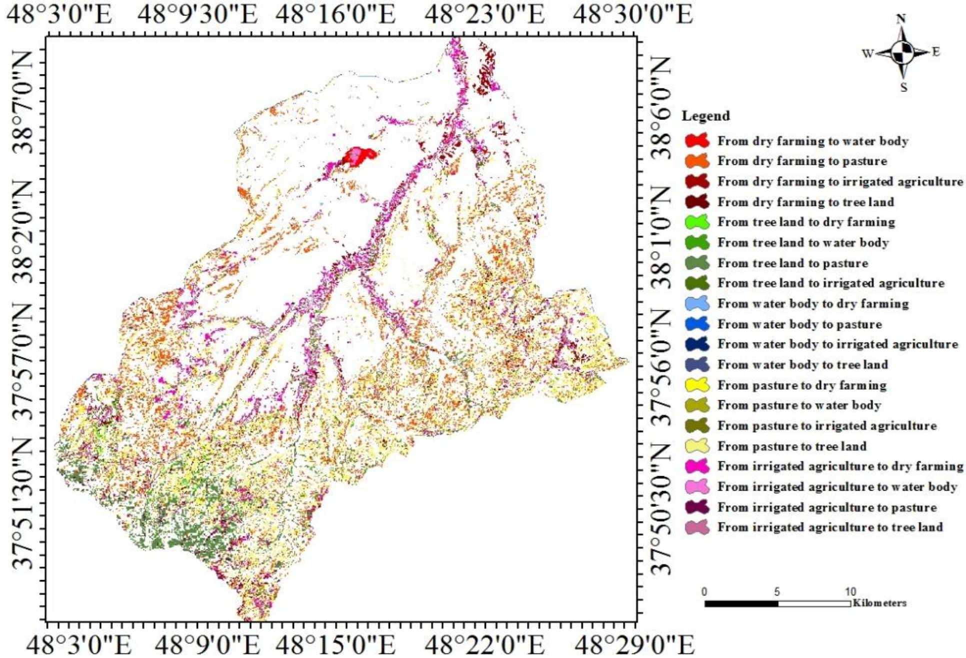


Figure 4. Land use changes thematic map for 1988-20

Table 3. Land use transition matrices statistics (%) during 1988-2000 in the study area

Land use	Dry farming	Water-body	pasture	irrigated agriculture	Tree land	Class total
Dry farming	82.655	88.443	10.670	43.667	14.866	100
Water-body	0.622	0.032	0.021	1.901	0.148	100
Pasture	12.100	0.052	83.166	8.621	43.422	100
Irrigated agriculture	2.987	0.109	0.413	28.382	5.518	100
Tree land	1.470	0.006	5.722	17.039	36.002	100
Built-up	0.167	0.000	0.008	0.390	0.034	100
Total	100	100	100	100	100	100
Changes	17.345	99.968	16.834	71.618	63.998	-

In 1988, the land use types in the Kozehtopraghi watershed included irrigated agriculture, dry farming, tree land, water-body, and pasture. By 2000, the land use types had changed to dry farming, irrigated agriculture, tree lands, water-body, pasture, and built-up. During the period 1988-2000, dry farming land use had changed by 17.345%, equaling an area of 82.39 km^2. Water-body land use had changed by 99.968%, equivalent to an area of 37.41km^2. Pasture had the lowest percentage of land use change in this period at 16.834%, with an area of 37.03 km2. Irrigated agriculture changed by 71.618% (41.92 km^2), while tree land had changed by 63.988%, equaling 8.876 km^2. Additionally, 0.167%, 0.008%, 390.0%, and 0.034% were changed from dry farming, pasture, irrigated agriculture, and tree land to built-up, respectively, with equivalent areas of 0.79 km^2, 0.018 km^2, 0.202 km^2, and 0.029 km^2.

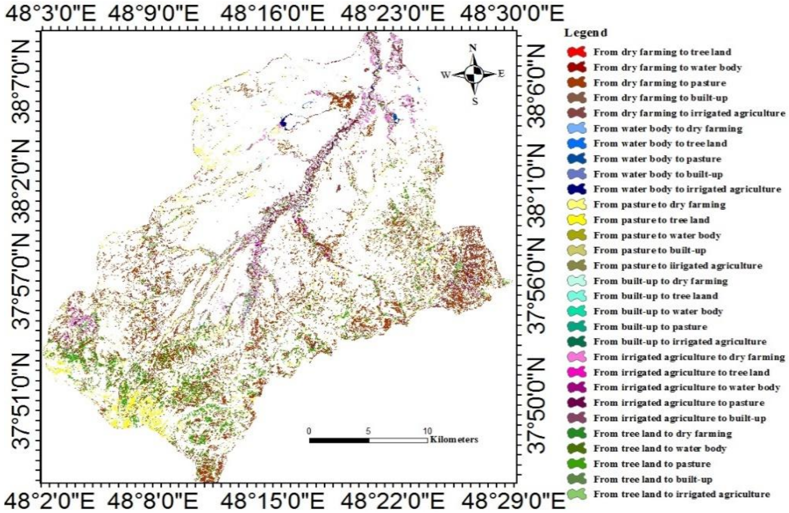


Figure 5. Land use change thematic map for 2000-2010 period

Table 4. Land use transition matrices statistics (%) during 2000-2010 in study area

Land use	Tree land	Water-body	pasture	Built-up	irrigated agriculture	Dry farming	Class total
Dry farming	8.906	12.792	6.304	42.584	28.897	23.481	100
Tree land	24.483	0.469	3.584	0.695	6.046	0.148	100
Water-body	0.089	53.293	0.118	7.961	0.579	69.240	100
pasture	51.803	6.327	88.962	7.550	12.761	5.318	100
Built-up	0.048	4.637	0.080	25.004	0.217	0.125	100
Irrigated Agri	15.671	22.483	0.951	16.206	51.500	1.721	100
Total	100	100	100	100	100	100	100
Changes	75.517	46.707	11.038	74.996	48.500	76.519	-

During the period 2000-2010, land uses in the Kozehtopraghi watershed included dry farming, irrigated agriculture, water-body, tree land, pasture, and built-up. According to the results of the 2000 to 2010 conversion matrix, dry farming, tree land, and built-up had changed by 76.519%, 75.517%, and 74.996%, respectively, with areas of 455.97 km^2, 51.521 km^2, and 1.998 km^2. In contrast, pasture, water-body, and irrigated agriculture had changed by 11.038%, 44.707%, and 48.500%, respectively, with areas of 27.77 km2, 2.45 km^2, and 34.13 km^2.

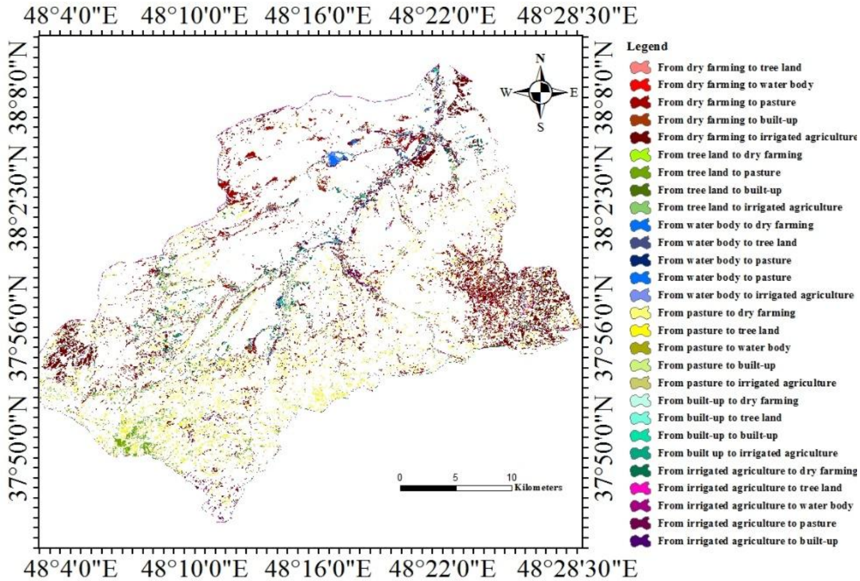


Figure 6. Land use change thematic map for 2010-2018 period

Table 5. Land use transition matrices statistics (%) during 2010-2018 in study area

Land use	Tree land	Water-body	pasture	Built-up	irrigated agriculture	Dry farming	Class total
Dry farming	1.362	0.144	9.171	35.881	29.912	23.530	100
Tree land	46.545	99.540	3.178	0.032	6.859	0.159	100
Water-body	0.000	0.043	0.001	0.000	0.001	0.002	100
pasture	23.118	0.083	69.932	23.531	16.359	8.710	100
Built-up	0.067	0.030	0.121	30.441	1.338	0.455	100
Irrigated Agri	28.907	0.160	17.596	10.115	53.533	8.433	100
Total	100	100	100	100	100	100	100
Changes	75.517	46.707	11.038	74.996	48.500	76.519	-

The land use conversion matrix for the period 2010-2018 shows changes of 76.519%, 75.517%, and 74.996% in dry farming, tree land, and built-up land uses, respectively. Pasture had the lowest land use change during this period, with a change of 11.038% or an area of 39.79 km^2.

Additionally, the land use change map of entire period (1988-2018) has been shown in Figure 7, and Table 6.

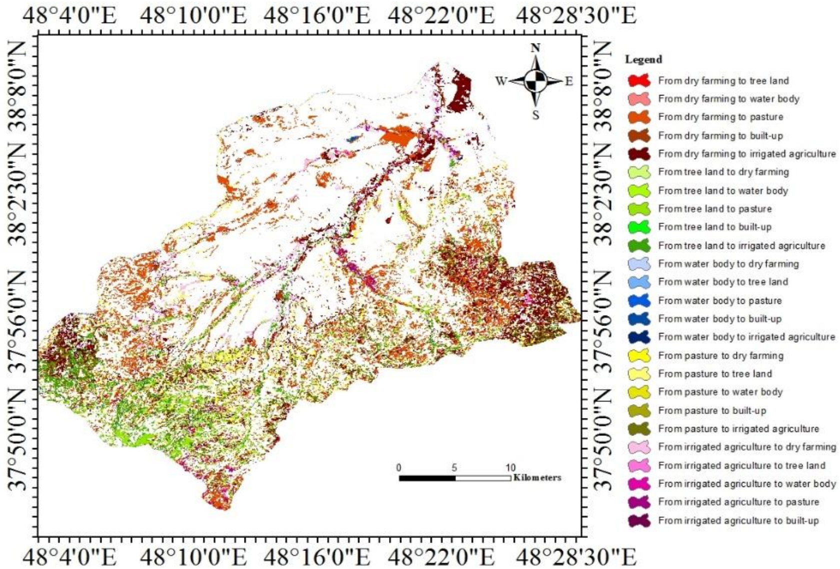


Figure 7. Land use change thematic map for 1988-2018 period

Table 6. Land use transition matrices statistics (%) during 1988-2018 in study area

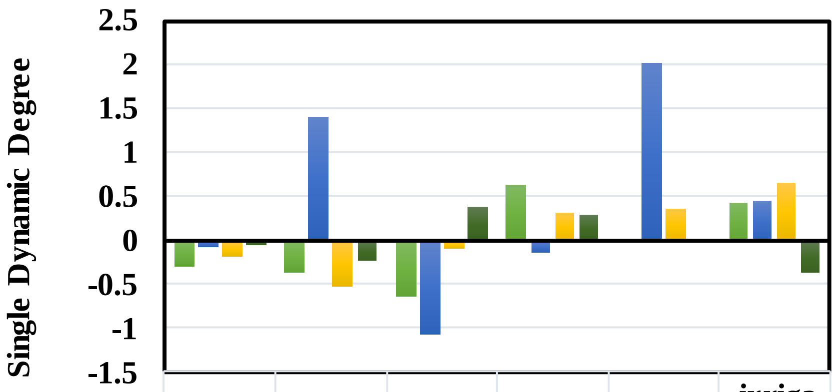
Land use	Dry farming	Tree land	Water-body	pasture	irrigated agriculture	Class total
Dry farming	66.687	7.232	0.088	8.573	26.043	100
Tree land	0.699	19.444	88.444	3.906	6.079	100
Water-body	0.066	0.005	0.003	0.181	8.621	100
pasture	20.485	36.755	0.057	73.000	23.271	100
Built up	0.523	0.473	0.005	0.157	1.441	100
Irrigated Agri	11.540	36.090	0.044	14.360	42.984	100
Total	100	100	100	100	100	100
Changes	33.313	80.556	99.997	27.000	57.016	-

Over the 30-year period in the Kozehtopraghi watershed, tree land, pasture, dry farming, and irrigated agriculture changed by 99.997% (9.19 km²), 80.556% (52.77 km²), 27% (59.998 km²), and 57.016% (29.48 km²), respectively. Additionally, 2.484 km² (0.523%) of dry farming, 0.31 km² (0.473%) of tree land, 0.058 km² (0.005%) of water-body, 0.35 km² (0.157%) of pasture, and 0.75 km² (1.441%) of irrigated agriculture were converted to built-up land.

In assessing land use dynamic changes, a positive value indicates a high intensity and rate of change in different land use classes, while a negative value signifies a lower intensity and speed of change (Wu *et al.*, 2023). The single land use change dynamics were calculated, and the results are shown in Figure 8.

The results of the single land use change dynamics over four periods (1988-2000, 2000-2010, 2010-2018, and 1988-2018) reveal that dry farming exhibited negative values in all periods, indicating a lower speed and intensity of land use change throughout the 30-year period in the Kozehtopraghi watershed. Dry farming consistently had negative values, indicating slow and low-intensity change over the 30-year period. Tree land exhibited high change intensity (1.408%) in 2010–2018 but declined in earlier periods (–0.237% in 1988–2000 and –0.45% in 2000–2010), resulting in an overall low-intensity decrease (–0.373%) over three decades. Water-body land use saw rapid change (0.371%) in 1988–2000 but slowed in later periods (–0.09% in 2000–2010, –1.064% in 2010–2018), leading to an overall low-intensity decline (–0.652%) by 2018.

Pasture land use showed high-intensity change during 1988–2000 (0.288%) and 2000–2010 (0.315%), but declined (-0.151%) in 2010–2018. Over the 30-year period, it had a positive change intensity of 0.626%. Built-up areas, analyzed from 2000 onwards, exhibited high change intensity (0.365% in 2000–2010 and 2.009% in 2010–2018). Irrigated agriculture had variable trends, with negative change (-0.374%) in 1988–2000 but positive values (0.658%, 0.442%) in later periods, resulting in an overall 0.425% increase.



	Dry farming	Tree land	water body	pasture	Built-up	irrigated agriculture
1988-2018	-0.299	-0.373	-0.652	0.626	0	0.425
2010-2018	-0.0722	1.408	-1.064	-0.151	2.009	0.442
2000-2010	-0.18	-0.54	-0.09	0.315	0.356	0.658
1988-2000	-0.0439	-0.237	0.371	0.288	0	-0.374

Figure 8. Single land use change dynamics in the study area

The integrated land use change dynamics were calculated using Equation 2, and the results are presented in Figure 9.

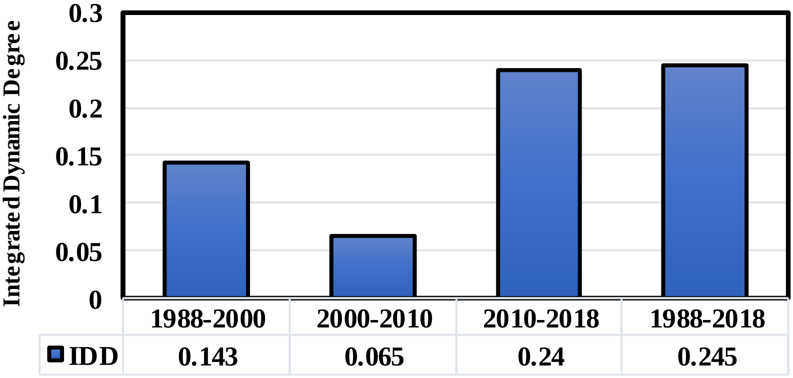


Figure 9. Integrated land use change dynamics in the Kozehtopraghi watershed over four periods

Single land use dynamic change was evaluated by analyzing each land use type separately over the study period, without combining all changes. For a broader perspective, integrated land use dynamic change was assessed by examining all land use changes together. The results showed that the 2010–2018 period had the highest integrated change rate (0.24%), indicating the most intense land use transformations. The rates for 1988–2000 and 2000–2010 were 0.143% and 0.065%, respectively. Over the entire 1988–2018 period, the land use change dynamics reached 0.245%. The land use dynamic degree values reflect the extent of change over time, with higher values signaling more significant shifts, such as urbanization or agricultural expansion. Interpretation depends on the study's context, including land use categories, time frame, and other relevant factors. Lower values suggest stability, while higher values indicate rapid transformation.

The findings of this study align with and expand upon previous research on land use change dynamics. For instance, Chunxiao *et al*. (2008) observed a shift from grassland and woodland to cultivated areas, similar to the significant conversion of pasture and tree land to agricultural and built-up areas in the Kozehtopraghi watershed. However, this study uniquely indicates the role of socioeconomic factors, such as low farmer income and the lack of mechanization, as key drivers of land use change, which were not explicitly addressed in earlier works. Sun *et al*. (2013) noted declining land use change intensity over time, whereas this study reveals a high intensity of change in built-up areas (2.009%) and tree land (1.408%) during 2010-2018, suggesting a more recent acceleration in urbanization and private garden expansion. Additionally, while Liu *et al*. (2018) reported high dynamics in artificial water bodies, this study identifies water-body changes as highly dynamic in 1988-2000 (0.371%) but declining thereafter, reflecting regional water resource depletion. The findings also contrast with Talebi Khiavi and Mostafazadeh (2021), who stated forest decline, as this study shows tree land dynamics increasing in recent years due to socioeconomic incentives like private gardening.

Single land use change dynamics

Analysis of single land use change dynamics across four periods (1988–2000, 2000–2010, 2010–2018, and 1988–2018) revealed that dry farming consistently had negative values, reflecting a slow and low-intensity change over the 30-year span in the Kozehtopraghi watershed. In contrast, tree land exhibited a high change rate (1.408%) during 2010–2018, likely driven by rising interest in private gardens, garden houses, and profitable garden-based income. However, it showed negative values (−0.237% and −0.45%) in 1988–2000 and 2000–2010, indicating slower change. Overall, tree land experienced low-intensity change (−0.373%) across the 30-year period. Water-body land use displayed positive values, peaking at 0.371% (high intensity and speed) in 1988–2000.

However, during 2000–2010, 2010–2018, and 1988–2018, land use change showed very low intensity, with negative values of -0.09%, -1.064%, and -0.652%, respectively. Pasture change had high intensity and speed in 1988–2000 and 2000–2010, with positive values of 0.288% and 0.315%, respectively, but a low intensity (-0.151%) in 2010–2018. Over the entire 30-year period, pasture change had a positive intensity (0.626%) and high speed. Build-up land use, not classified in 1988, showed high change intensity and speed in 2000–2010 (0.365%) and 2010–2018 (2.009%). Conversions to built-up included 0.167% (0.792 km^2) from dry farming, 0.008% (0.018 km^2) from pasture, 390.0% (0.2016 km^2) from irrigated agriculture, and 0.034% (0.0288 km^2) from tree land. Irrigated agriculture exhibited positive change intensities of 0.658% and 0.442% in two periods but a negative value (-0.374%) in 1988–2000. Over the 30-year span, it had a positive intensity (0.425%), indicating high land use change.

Integrated land use change dynamics

This study explores the integrated dynamics of land use change by quantifying shifts in various land uses, focusing on the area of change during the study period. The period from 2010-2018 had the highest dynamic change of 0.24% for all land uses, while the periods of 1988-2000 and 2000-2010 had values of 0.143% and 0.065%, respectively.

Driving forces of the land use change dynamic

Land use is significantly shaped by changes in agricultural practices, which in turn impact management and economic strategies (Briassoulis, 2009). The swift expansion of land use changes is contributing to the degradation of arid regions, with fertile scrub soils being particularly vulnerable to erosion and destruction, especially in densely populated areas. It is evident that land with low vegetation is subject to runoff destruction and poor water retention (Meshesha *et al.*, 2016; Spalevic *et al.*, 2013). The evolution and changes of land use in a particular area reflect the natural and socioeconomic aspects of the area and their application over time and space. Assessing land use change helps identify the extent of human impacts on the environment (DeFries *et al.*, 2004).

Understanding land use change and land use status at temporal and spatial scales is critical for environmental management (Sreenivasulu, 2014). Declining soil fertility and inadequate mechanization have reduced agricultural incomes, motivating land use changes and farmland expansion. Socioeconomic factors predominantly drive short-term transformations, particularly in the Kozehtopraghi watershed, where pasture conversion to croplands and orchard expansion occur due to income pressures and higher profitability. Weak governance exacerbates these changes, as ineffective laws fail to prevent pasture degradation, leading to: (1) reduced livestock productivity and (2) abandoned lands vulnerable to erosion. Poor enforcement of land use regulations and legal loopholes further enable unsustainable practices.

Regarding the drivers of land-use dynamics, government policies promoting agricultural expansion and subsidies for certain crops may have incentivized farmers to convert pasture and forested areas into farmland. Additionally, demographic changes, such as population growth and rural-urban migration, likely intensified pressure on land resources. Economic conditions, including fluctuating market prices for agricultural products, may have also influenced land-use decisions, with farmers shifting to more profitable crops or abandoning less productive lands. The lack of stringent land-use regulations and enforcement mechanisms further exacerbated these changes. The absence of policies to protect pasturelands from plowing or to regulate urban sprawl allowed for unchecked land-use transitions. Moreover, the growing trend of private garden ownership and the economic benefits derived from orchard activities likely contributed to the expansion of tree land, particularly in the 2010-2018 period. These socioeconomic dynamics, combined with limited access to modern agricultural technologies and declining soil fertility, created a feedback loop that perpetuated land-use changes. To better understand these influences, future research could incorporate interviews with local stakeholders, analysis of policy documents, and economic data to identify specific drivers and their impacts. This would provide a more nuanced interpretation of the observed land-use transitions and their environmental consequences, such as soil degradation, loss of biodiversity, and reduced water availability. By linking these socioeconomic factors directly to land-use changes, the study can offer more actionable indications for policymakers aiming to balance economic development with environmental sustainability in the region.

Over the 30-year study period (1988-2018), significant land-use changes were observed in the Kozehtopraghi watershed. Dry farming, the dominant land use, experienced a 17.3% reduction in area by 2000, with further declines in subsequent periods, reflecting a shift away from traditional agricultural practices. Tree land saw notable fluctuations, with a sharp increase in the 2010-2018 period (1.4% annual change), likely driven by economic incentives for private gardens and orchard expansion. However, pastureland declined significantly, particularly between 1988 and 2000, with a 16.8% reduction, leading to increased soil erosion and reduced water r rapidly, especially between 2010 and 2018, with a 2% annual increase, indicating urbanization pressures. The most significant transitions included the conversion of pasture and dry farming to built-up areas and tree land, driven by socioeconomic factors such as low agricultural income, population growth, and weak land-use regulations. These changes have had profound environmental consequences, including soil degradation, loss of biodiversity, and reduced water availability. The expansion of tree land, while economically beneficial, has also contributed to the fragmentation of natural habitats. Overall, the study proves the need for sustainable land-use planning to balance economic development with environmental conservation in the Kozehtopraghi watershed.

Management and poliy implications:

The study found increasing land use changes in recent periods, driven by farmers illegally converting pastures to croplands for economic gain. Shifting rural lifestyles, with fewer youths willing to engage in pastoral farming, further boosted agricultural expansion. Drought, declining pasture productivity, and rising livestock costs also reduced pastoral activities. The trend suggests continued land use intensification. These findings can guide policies to control unsustainable changes and protect ecological balance. Future research should incorporate socio-economic and climate data for better land management strategies.

- To mitigate negative land-use impacts, policymakers should implement strict zoning laws to protect vulnerable pastures while offering financial incentives like subsidies for sustainable farming practices to reduce economic pressures.

- Strengthening enforcement of anti-degradation laws and penalizing illegal conversions should be prioritized alongside promoting agroforestry and integrated crop-livestock systems to balance productivity with ecological preservation.

- Government-led programs should provide mechanized farming training to improve yields without expansion, while community-based initiatives could empower local stakeholders to monitor land-use changes effectively.

- Restoration projects for degraded pastures should be implemented with rotational grazing incentives, and climate-smart agriculture (e.g., drought-resistant crops, efficient irrigation) must be adopted to enhance resilience.

- A regional monitoring system integrating remote sensing with ground surveys would enable timely interventions, complemented by urban planning policies that restrict development on fertile lands.

CONCLUSIONS

In this study, the combination of remote sensing and GIS was used to identify the pattern of land use changes, as well as to investigate the dynamics of land use change in the Kozehtopraghi watershed over a 30-year period. Additionally, the extent of changes in different land uses was evaluated using the land use dynamic degree approach, employing both single and integrated indices. The land use change dynamic can be used to quantify the degree of change in land use patterns within a given area, such as a region or watershed. Over 30 years in the Kozehtopraghi watershed, dry farming showed negative single dynamics, tree land peaked at 1.408% during 2010-2018, water-body use declined after a 0.371% peak in 1988-2000, pasture use decreased after 2010, build-up land rapidly expanded post-2000, and irrigated agriculture showed overall positive change. According to integrated dynamic degree, this study quantifies land use changes, peaking at 0.24% during 2010-2018, compared to 0.143% in 1988-2000 and 0.065% in 2000-2010. Socioeconomic factors drive land use changes in the Kozehtopraghi watershed, with low farmer incomes leading to pasture plowing and agricultural expansion, while income from gardening promotes garden area growth. Weak management and legal enforcement exacerbate these changes, resulting in pasture degradation, reduced animal husbandry income, increased runoff, and soil loss. Land use dynamics in the study area have intensified recently, driven by economic factors, such as illegal pasture conversion, changing rural lifestyles, drought, and rising animal husbandry costs. The observed trend suggests continued intensification, indicating the need for effective planning to control land use changes and maintain ecological integrity, with future research benefiting from broader temporal and socioeconomic analyses.

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YIELD AND QUALITY OF GRAPES FROM MONTENEGRIN SEEDLESS VARIETIES IN THE PODGORICA SUBREGION

SUMMARY

This study presents the results of research on the agrobiological, economic and technological characteristics of Montenegrin seedless grapevine varieties grown in the ampelographic collection of the Biotechnical Faculty in Podgorica. The research was conducted in 2020–2021 period. The highest grape yield and cluster weight were recorded in the variety PIT 15 (2.23 kg/m² and 428 g), while the lowest values were observed in the variety Razaklija Besjemena (1.45 kg/m² and 225 g). The variety PIT 15 also exhibited the highest average berry weight (3.23 g), whereas the smallest berries were measured in the variety Podgorička Besjemena (2.64 g). The sugar content in grape juice varied significantly among the studied varieties. The highest sugar levels were recorded in varieties PIT 14 (16.2%) and Podgorička Besjemena (16.1%), while the lowest sugar levels were observed in PIT 15 and Razaklija Besjemena (15.3%). The total acidity of the grape juice was generally low during both years of the study, which is characteristic of Mediterranean climatic conditions such as those in Montenegro. The highest average acidity over the two years was found in the variety PIT 14 (5.40 g/L), while the lowest acidity was recorded in PIT 15 (4.70 g/L). Statistical analysis revealed that the differences among all the parameters studied were highly significant.

Keywords: grapevine, seedless varieties, yield, cluster weight, grape quality

INTRODUCTION

Global grape production reaches approximately 75 million tons annually, making grapevines one of the most widely cultivated fruit crops in the world (Akkurt, 2019; Kaya, 2020; Boztepe, 2023). Of the total grape production, the majority is used for winemaking, while a smaller portion is consumed fresh, dried, or processed into various products (Bešlić, 2019; Izcara, 2021).

The technology for producing table grapes for large markets and export has been nearly perfected. This is increasingly significant given rising living

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standards and growing consumer expectations regarding the nutritional content and quality of grapes (Al Saif, 2022). Today, consumers have access to fresh grapes of impeccable appearance year-round, sourced from various countries around the world.

In recent decades, seedless grape varieties have become highly popular and valued by consumers, gradually replacing seeded varieties (Royo et al., 2018). The primary use of seedless grape varieties has historically been for producing raisins, but their importance as table grapes for fresh consumption has grown significantly in recent years (Matijašević, 2021). However, seedless varieties also have certain limitations, including small berry size, fragile pedicels, and a firm attachment of berries to the stem. To produce high-quality table grapes with larger berries from these varieties, special practices are employed, such as cane or trunk girdling, treatment with growth regulators, and other methods (Cindrić et al., 2000, 2003; Korać, 1998; Dimovska et al., 2014).

The world's largest producers of raisins are California, contributing approximately 30% of global raisin production, followed by Turkey (23%), Greece (21%), Australia (10%), and Iran (6%) (Cindrić et al., 2019; Bešlić, 2019). Smaller quantities of raisins are also produced in Argentina, Chile, Italy, and Morocco.

The Podgorica wine-growing region, characterized by its Mediterranean climate, provides ideal conditions for cultivating table grape varieties of all ripening epochs (Popović, 2024). However, despite favorable agroecological conditions, table grape production is significantly less prevalent compared to wine grape varieties. Of the total vineyard area in Montenegro (2,800 ha), only 7.89% is dedicated to table grape varieties (Rejonizacija). Furthermore, seeded table grape varieties dominate these areas, while seedless varieties remain underrepresented and are mostly studied in ampelographic collections.

At the Experimental Field of the Biotechnical Faculty in Podgorica, several seedless grapevine varieties of diverse origins are cultivated, including five seedless varieties developed at the Agricultural, later Biotechnical Institute in Podgorica.

The aim of this study was to examine and present in detail the agrobiological and economic-technological characteristics of these five seedless grapevine varieties (Podgorička Besjemena, Razaklija Besjemena, PIT 14, PIT 15, PIT 16) to provide more data to science and practice, enabling better evaluation, more intensive cultivation, and broader dissemination of these varieties in the viticultural regions of Montenegro and beyond.

MATERIAL AND METHOD

Vineyard location

The study of agrobiological, economic and technological characteristics of Montenegrin seedless grapevine varieties was conducted during the 2020 and 2021 growing seasons. The research was performed in the Collection Vineyard of the Biotechnical Faculty in Podgorica (42° 26′ 78″ N, 19° 12′ 57″ E), established in 2005 with a planting distance of 2.4 m between rows and 1 m within row. The vines were trained as double-arm horizontal cordons with a trunk height of approximately 80 cm. Mixed pruning was applied. The experimental vineyard was irrigated using a drip irrigation system throughout the study. The trials included 75 vines in total, organized into three replications with five vines per replication.

Description of Studied Varieties

The following varieties were evaluated: Podgorička Besjemena (PB), Razaklija Besjemena (RB), PIT 14, PIT 15, and PIT 16.

Podgorička Besjemena was developed by crossing Bijeli Čauš with Perlette . It produces medium-sized clusters and berries with a light green to golden-yellow skin and ripens in early August (Figure 1)

Figure 1. Podgorička besjemena

–Razaklija Besjemena was created by crossing Razaklija with Sultanina. It inherited the cluster and berry shape of Razaklija and seedlessness from Sultanina. Ripens in late August.

–PIT 14 is an early ripening seedless variety (mid-August) resulting from the cross of Bijeli Čauš and Perlette. It features medium-sized clusters and berries with a yellow-green color and muscat flavor.

–PIT 15 is a late-season variety that ripens in early September. It was developed by crossing Razaklija and Sultanina to create a high-yielding seedless variety with loose, large clusters and medium-sized greenish-yellow berries.

–PIT 16 was developed from a cross between Bijeli Čauš and Sultanina. It is characterized by large, loose clusters and oval-shaped greenish-yellow berries with an aromatic and pleasant flavor. This high-yielding variety also ripens in early September.

Evaluation of Agrobiological Parameters and Analytical Methods Used in the Study

During the two-year study, the following parameters were assessed: the number of clusters, grape yield (kg/m²), cluster and berry mass (g), cluster and berry length and width (cm for clusters, mm for berries), and sugar content (%) and total acidity (g/L) in grape juice. Grape yield was determined by weighing the harvested grapes, while cluster mass was calculated based on the yield from five vines and the total number of clusters. After harvest, the length and width of clusters and berries, as well as the average berry mass, were measured. Sugar content in grape juice was determined areometrically with Oechsle hydrometer. Total acidity in grape juice was measured through neutralization of all acids and their salts using a 0.1N NaOH solution, with bromothymol blue as the pH indicator.

The collected data were analyzed using analysis of variance (ANOVA) for a completely randomized block design. The significance of differences was determined using the LSD test.

Climate Conditions at the Experimental Site

Climatic analysis for the Lješkopolje region was based on data from the meteorological station in Podgorica. The average annual and growing season air temperatures during the study years were relatively consistent (Figure 2). The average annual and growing season air temperatures in 2020 were slightly higher (17.2°C and 22.4°C) compared to 2021, when they measured 17.0°C and 22.3°C, respectively. The sum of effective temperatures was notably high (2669°C and 2636°C), classifying this wine-growing region into Zone V according to Winkler's classification, indicating a very warm climate.

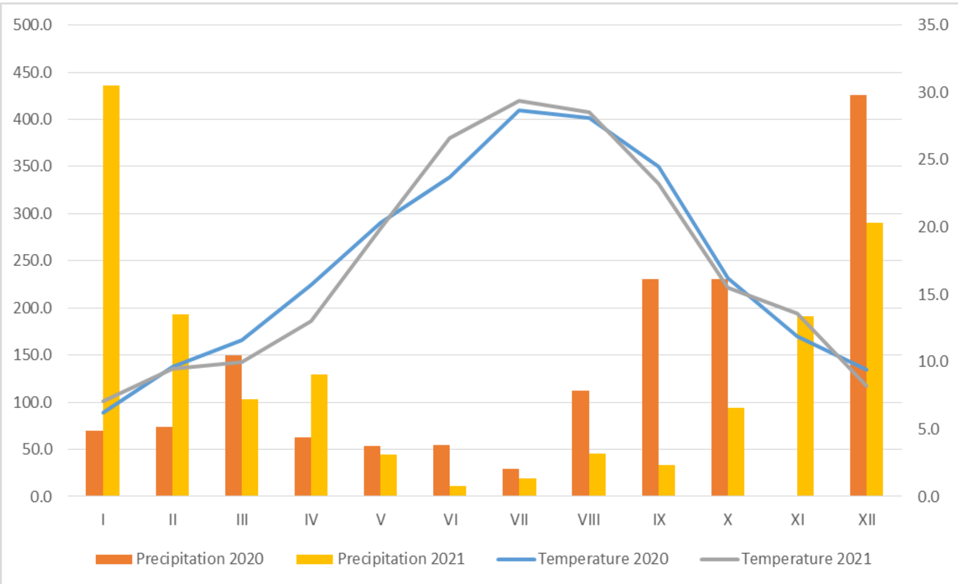

Figure 2. Average monthly precipitation and temperatures during the 2020-2021 period.

The annual precipitation sum in 2021 was higher, resulting in 1590.5 l/m². However, during the growing season, more precipitation occurred in 2020 (775.0 l/m²). Notably, the period from August to September 2020 experienced particularly heavy rainfall, with 112 l/m² in August and 231 l/m² in September.

RESULTS AND DISCUSSION

Based on the data in Table 1, it can be observed that the number of clusters in the studied period ranged from 13 to 15.8 for the 2020 harvest and from 12 to 15.1 for the 2021 harvest. In terms of average values for the two studied harvests, the highest number of clusters was found on the Razaklija Besjemena vines (15.5), while the lowest number was observed in the PIT 15 variety (12.5). Statistical analysis revealed a significant difference in the number of clusters between Razaklija Besjemena and all other studied varieties.

The cluster size, as a very important parameter for the quality of table grapes, was determined based on the average mass, length, and width of the cluster. Analyzing the two-year average values of cluster mass, it can be observed that the largest cluster mass was found in the varieties PIT 15 (428 g) and PIT 16 (377 g), while the smallest mass was recorded for the Razaklija besjemena variety (225 g). Statistical analysis showed that the PIT 15 and PIT 16 varieties had significantly higher cluster mass compared to all other studied varieties. A significant difference in cluster mass was also observed between the Podgorička besjemena and PIT 14 varieties when compared to the Razaklija besjemena variety. The average cluster mass for all studied varieties was higher in the first year of research (331g) compared to the second year (307g), which is likely due to the higher precipitation amounts during the May-July period of 2020, when the berries were growing intensively. These results align with those of Popović et al. (2013), who found that the cluster mass of the Vranac variety in the Podgorica wine region was also higher in climate-favorable years. The average cluster mass of the PIT 15 variety in these studies was in line with the values reported by Ulićević et al. (1991) under the same agroecological conditions, while the cluster mass of the PIT 14 and PIT 16 varieties was slightly lower than the results from their research.

Table 1. Cluster weight and grape yield of studied varieties

Variety	Number of clusters		Average	Cluster weight (g)		Average	Grape yield (kg/vine)		Average
	2020	2021		2020	2021		2020	2021	
PB	14.0	13.0	13.5	314	292	303	1.83	1.58	1.70
RB	15.8	15.1	15.5	239	211	225	1.57	1.33	1.45
PIT 14	14.6	13.5	14.0	275	248	261	1.68	1.40	1.54
PIT 15	13.0	12.0	12.5	435	420	428	2.36	2.10	2.23
PIT 16	14.0	13.0	13.5	393	362	377	2.29	1.96	2.12
Average	14.2	13.6	13.9	331	307	319	2.06	1.67	1.81

Parameter	Number of clusters		Cluster weight		Grape yield	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2020-2021	0.628	0.872	16.998	23.577	0.174	0.241

Grape yield per unit area, as an absolute indicator of variety productivity, depends on various factors such as the genetic potential of the variety, production technology, vineyard age, climatic conditions, and the health of the vines (Melo & Ribeiro, 2011; Popović, 2012). Based on the results in Table 1, significant variations in grape yields were observed between the studied varieties and the years of research. The yields in the studied years ranged from 1.57 kg/m² to 2.36 kg/m² in 2020 and from 1.33 kg/m² to 2.10 kg/m² in 2021. The lowest grape yields over the two-year average were observed for the Razaklija besjemena and PIT 14 varieties (1.45 kg/m² and 1.54 kg/m²), while the highest yields were recorded for the PIT 15 variety (2.23 kg/m²) and PIT 16 variety (2.12 kg/m²). Statistical analysis revealed that the grape yield for the PIT 15 and PIT 16 varieties was significantly higher over the two-year average compared to all other studied varieties, while the yield for the Podgorička besjemena variety was significantly higher only compared to the Razaklija besjemena variety. Other observed differences in yields were not statistically significant. The yields achieved in the studied years were similar to those reported by other authors for the studied varieties under the same agroecological conditions (Ulićević, 1991; Pejović, 1997, 2002).

Regarding the length and width of clusters for the studied varieties (Table 2), it was notable that the PIT 16 variety had the largest cluster length (20.6 cm) and width (12.9 cm), while the Razaklija besjemena variety had the smallest cluster length (16.0 cm) and width (10.6 cm).

Table 2. Length and width of cluster of studied varieties

Variety	**Cluster length (cm)**		**Average**	**Cluster width (cm)**		**Average**
	2020	2021		2020	2021	
PB	17.2	16.5	16.8	13.0	12.7	12.8
RB	16.3	15.8	16.0	11.3	9.9	10.6
PIT 14	16.1	15.9	16.0	11.6	10.6	11.1
PIT 15	20.2	19.1	19.6	12.3	11.8	12.0
PIT 16	22.3	19.0	20.6	13.5	12.3	12.9
Average	18.2	17.5	17.8	12.0	11.8	11.9

Parameter	**Cluster length**		**Cluster width**	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2020-2021	0.743	1.030	0.604	0.839

A statistically significant difference in cluster length was observed between the PIT 16 variety and all other studied varieties. Additionally, a significant difference in cluster length was noted between the Podgorička besjemena variety and the Razaklija besjemena and PIT 14 varieties. The cluster width was significantly larger in the PIT 16 and Podgorička besjemena varieties compared to all other studied varieties, and in the PIT 15 variety, it was larger only compared to the

Razaklija besjemena and PIT 14 varieties. Similar results regarding the cluster length and width of these varieties were obtained by Pejović (1997; 2002) under the agroecological conditions of the Podgorica subregion.

The data presented in Table 3, indicate that the berries of the PIT 15 variety had the greatest length (20.50 mm), while the shortest berry length was observed in the Podgorička besjemena variety (15.73 mm). Statistical analysis revealed that the PIT 15 and PIT 16 varieties had significantly larger berry lengths compared to all other varieties, while the PIT 14 and Razaklija besjemena varieties had significantly larger berry lengths only compared to the Podgorička besjemena variety.

As for the berry width, the largest berries were found in the PIT 14 variety (17.25 mm), while the smallest were in the Podgorička besjemena variety (13.38 mm). The difference in berry width was statistically significantly larger between the PIT 14, PIT 15, and PIT 16 varieties compared to the Razaklija besjemena and Podgorička besjemena varieties, as well as between the Razaklija besjemena variety and the Podgorička besjemena variety.

Table 3. Physical characteristics studied varieties

Variety	Berry length (mm)		Average	Berry width (mm)		Average	Berry weight (g)		Average
	2020	2021		2020	2021		2020	2021	
PB	16.33	15.13	15.73	13.70	13.07	13.38	2.72	2.57	2.64
RB	17.50	16.15	16.82	15.30	14.35	14.82	2.83	2.65	2.74
PIT 14	17.70	16.90	17.30	17.30	17.21	17.25	2.86	2.73	2.79
PIT 15	20.70	20.30	20.50	16.62	16.26	16.44	3.32	3.15	3.23
PIT 16	20.38	20.01	20.19	16.40	16.00	16.20	2.88	2.67	2.77
Average	18.52	17.69	18.10	15.87	15.37	15.62	2.92	2.75	2.84

Parameter	**Berry length**		**Berry width**		**Berry weight**	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01

For all GNSS measurements (static/rapid static), the data recording interval was set at 1 second and the elevation mask was utilized as 7.5°. The evaluation of static and rapid static GNSS data was conducted using Topcon Magnet Tools 8.1.0 commercial software, and precise positioning was achieved through the utilization of precision ephemeris and Final IGS products. The objective of this experiment was to compare the accuracy of different measurement techniques and to analyses methods to enhance positioning accuracy in forest areas. The experiments were conducted in selected forest areas in the Davutpaşa Campus region of Yıldız Technical University.

Two different geometries were selected for this purpose, where the angle (ɣ) was small (Figure 3) and around 100g (Figure 4). Initially, on 3 October 2023, points P1 and P2 were established in proximity to test site 1, while P5 and P6 were established in proximity to test site 2. GNSS measurements were then performed on these points in static measurement mode for duration of 2 hours, after which their coordinates were calculated.

Table 4. Chemical characteristics of studied varieties

Variety	**Sugar content**		**Average**	**Acid content**		**Average**
	2020	2021		2020	2021	
PB	15.7	16.5	16.1	5.5	5.0	5.2
RB	14.9	15.7	15.3	5.4	4.5	4.9
PIT 14	16.0	16.5	16.2	5.8	5.0	5.4
PIT15	14.7	16.0	15.3	4.3	5.1	4.7
PIT16	15.2	15.5	15.7	5.5	5.2	5.3
Average	15.3	16.0	15.7	5.2	5.1	5.1

Parameter	**Sugar content**		**Acid content**	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2020-2021	0.459	0.637	0.345	0.479

A significant difference was also found between the PIT 16 and Razaklija besjemena varieties. The sugar content in the juice was higher in all examined varieties in 2021, which is a direct consequence of the different meteorological conditions in the years the study was conducted, particularly the lower rainfall during the 2021 growing season.

The acidity content in grape juice is an important indicator of grape quality, as it affects the taste and harmony of the fruit (Popović, 2020). The highest acidity was found in the PIT 14 (5.40 g/l) and PIT 16 (5.30 g/l) varieties, while the lowest acidity was in the PIT 15 variety (4.70 g/l). Statistical analysis revealed that the PIT 14, PIT 16, and Podgorička besjemena varieties had significantly higher acidity content compared to the PIT 15 variety. Additionally, a significant difference was found between the PIT 14 and PIT 16 varieties compared to the Razaklija besjemena variety. The results of these two-year investigations showed that the acidity of grape juice was low during both years of the study, which is typical for Mediterranean climates such as that of Montenegro (Pajović, 2014).

CONCLUSION

Based on the conducted research, the following conclusions can be drawn:

–The average grape yield over the two-year period was highest for the PIT 15 variety (2.23 kg/m²), while the lowest yield was observed for the Razaklija besjemena variety (1.45 kg/m²).

–The number of clusters varied significantly among the tested varieties, ranging from 12.5 for PIT 15 to 15.5 for Razaklija besjemena.

–The average cluster mass ranged from 225 to 428 g, with the highest cluster mass found in the PIT 15 variety and the lowest in Razaklija besjemena.

–The average berry mass, length, and width were largest in the PIT 15 variety (3.23 g), while the Podgorička besjemena variety had the smallest berries (2.64 g).

–In the two-year average, the highest sugar content was found in PIT 14 (16.2%) and Podgorička besjemena (16.1%), while the lowest sugar content was in PIT 15 and Razaklija besjemena (15.30%). Sugar content was higher in all examined varieties in 2021, due to lower rainfall during the growing season of that year.

–The acidity content in the juice was characteristic for the varieties studied in the Podgorica vine region, with the highest acidity (5.4 g/l) found in PIT 14.

–All studied varieties, based on the two-year results, deserve greater attention and representation in the vineyards of Montenegro and beyond.

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A NEW METHODOLOGY FOR THE USE OF CORS TECHNIQUE IN THE FORESTED AREAS

SUMMARY

In forested areas, determining precise coordinates is difficult because dense vegetation and multi-path effects block Global Navigation Satellite System (GNSS) signals. This study proposes a new method to obtain coordinates without making direct GNSS measurements at the point of interest in the forest. Instead, the coordinates of the desired point are calculated theoretically using auxiliary points created in open areas. The positions of the auxiliary points are determined using Continuously Operating Reference Station (CORS)-based measurements. In this context, studies were carried out in two different test fields with different geometric configurations on the Davutpaşa campus of Yıldız Technical University. The point coordinates obtained from static, rapid static and CORS-based GNSS methods were compared with the reference coordinates obtained from total station measurements. The results showed that static GNSS provided the highest accuracy with the lowest deviation values from the reference data, while CORS had the lowest accuracy. However, all GNSS-based methods provided an accuracy of 10-15 cm, which was considered an acceptable level for forestry applications in this study. The accuracy of theoretical coordinates was affected by the geometric structure of the auxiliary points and the γ-angle between the two measurement lines. Namely, it leads to higher errors at small angles and around 200g angles. The proposed method provides a practical and efficient alternative for positioning in forested areas, reducing the long measurement times caused by GNSS signal occlusions, while maintaining sufficient accuracy for forestry and land surveying applications.

Keywords: CORS, multipath, forested area, coordinate computation, sine theorem

INTRODUCTION

The forestry industry's primary objective is the efficient utilization of forest resources. The meticulous planning and robust management strategies that underpin the monitoring and utilization of these resources are of paramount

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importance. In this context, the Global Navigation Satellite System (GNSS) plays a pivotal role, finding application in a variety of domains including forest road construction, damage monitoring in the context of illegal settlements or fires, forest harvesting and pest control (Tang et al. 2015).GNSS This technology facilitates the precise location of points, even in challenging environments such as forested areas, where traditional positioning systems encounter difficulties (Picchio, 2024; Zimbelman and Keefe 2018). However, numerous challenges arise in the context of positioning in forested areas, particularly in the presence of multipath effects, where signals experience attenuation due to the complex topography of the tree canopy (Cho, 2023; Feng et al. 2021; Cho et al. 2022).

Whilst terrestrial measurement methods have been shown to provide more accurate results than other methods, the challenges associated with their implementation in wooded areas limit their effectiveness (Cho et al. 2022). The performance of GNSS-based positioning in forested environments is significantly impacted by environmental conditions and limitations inherent in the method. For instance, static GNSS measurements have been observed to reduce positioning performance by extending the integer ambiguity resolution time when signals are blocked or distorted (Gallo, 2013). Consequently, the development of fast and precise positioning techniques for forested areas remains a significant research priority.

Real-Time Kinematic (RTK) GNSS measurements are utilized extensively due to their capacity to deliver centimeter-level precision and immediate positioning. The CORS technique further augments this capacity, enabling high-accuracy positioning with a solitary receiver and obviating the necessity for multiple crews and GNSS receivers (Wells and Chung 2023). Nevertheless, when the conditions surrounding the measurement change (e.g., in forest areas), the accuracy offered by the CORS technique may not be consistently achieved (Brach et al. 2019). Uzodinma and Nwafor (2018) reported that GNSS measurements may be unreliable in dense canopies. The accuracy of the CORS technique is significantly affected by the propagation of low-power, high-frequency GNSS signals, and signals blocked by intervening tree canopies, which have been shown to have a substantial impact on positioning accuracy (Catania et al. 2020; Kim et al. 2023; Feng et al. 2021; Yan et al. 2021).

A large number of studies have been conducted with the objective of enhancing the precision of GNSS measurements in wooded areas. For instance, Brach (2022) conducted rapid static measurements at points constituting a 33-point network in a forested region, achieving accuracies of 1.38/1.29 m and 0.74/0.91 m using GIS and geodetic-class GNSS receivers, respectively. In a similar vein, Bakuła et al. (2015) evaluated the performance of rapid static measurement methods employing multiple GNSS receivers, utilizing a bespoke apparatus in forested environments, observing enhancements in accuracy from a few centimeters to meters. Moreover, Brach and Zasada (2014) employed extended antennas to mitigate multipath effects in forested environments (Karjalainen, 2023). Pirti et al. (2016) achieved an accuracy of 6.5 cm with the CORS measurement technique near forested areas, demonstrating the potential for high accuracy positioning even in challenging conditions (Abdi et al. 2022).

This study proposes a novel and practical measurement method that aims to determine point coordinates in forest areas within a time frame of 3-5 minutes. To this end, four points forming two lines were marked in areas with clear visibility around the forest, in the same direction as the point to be determined in the forest. CORS measurements were made at these four points, and their coordinates were obtained. Utilizing these coordinates, the coordinates of the points were determined as a post-process, without the necessity of making measurements at the point in the forest area (points T_1 and T_2).The motivation of this study is to determine the coordinates of the points to be located in the inner areas of the wooded areas close to the open areas in a short time, such as 3-5 minutes. This approach ensures the location is determined with an accuracy of a few centimeters, mitigating the multipath and signal blocking issues commonly encountered in GNSS measurements in such environments.

MATERIAL AND METHODS

GNSS surveys

The static GNSS measurement method is utilized for high-accuracy positioning, particularly in forested areas where environmental conditions are challenging. This method involves installing the GNSS receiver at the designated point and collecting data over an extended period, thereby enhancing signal quality and minimizing atmospheric effects and other error sources (Zimbelman and Keefe, 2018; Xin et al. 2018). The collected data are processed with post-processing techniques, with reference station data and precise ephemeris information being used to correct positioning errors and ensure the obtained coordinates offer higher accuracy compared to other GNSS measurement methods (Xu et al. 2021). The accuracy of measurement is contingent on the quality of the receiver employed, the duration of the measurement, and environmental factors. In forested areas, elements such as tree canopy can adversely affect signal transmission, necessitating the judicious selection of measurement points (Weaver et al. 2015; Geng et al. 2020). This method is of great significance for land measurement, construction projects, environmental monitoring, especially the management of forest resources (Yeh, 2025; Yang et al. 2022).

The rapid static measurement method is a technique that allows high-accuracy location acquisition with short-term observations based on GNSS technology (Alkan et al. 2015). It requires shorter observation times (usually 3-5 minutes) compared to traditional static measurement, which provides a great advantage, especially in applications with time constraints. The GNSS receiver gathers and archives satellite signals during the measurement period, and post-processing techniques ensure minimal location errors (Geng et al. 2020). This method has been employed in all domains where static measurement is utilized, and it is notable for its capacity to deliver reliable and precise location in a brief timeframe (Wu et al. 2019).

The CORS system has a wide range of uses as a reference network that provides high accuracy and reliability. This system, which consists of geographically distributed stations, continuously monitors GNSS signals and provides users with real-time or post-processed correction data (Dardanelli et al.

2022; Pehlivan et al. 2019). The CORS system has been shown to provide decimeter or sub-decimeter precision by minimizing factors that affect positioning accuracy, such as atmospheric delays and satellite signal errors (Pipitone, 2023; Wu et al. 2015). When integrated with real-time kinematic (RTK) technology and various differential correction methods, the CORS system plays a pivotal role, particularly in fields such as land surveying, civil engineering, environmental monitoring, and disaster management (Öğütcü, 2020; Tran et al. 2023). The wide area coverage it provides enables users to achieve high-accuracy location even at longer distances. However, it is most widely preferred in engineering and scientific research due to its data continuity and reliability (Yurdakul and Kalayci, 2022; Liu et al. 2019).

Description of the Experiment

This study proposes a methodology for the implementation of the positioning method in forest areas where GNSS signals are not suitable for multipath or signal blocking measurements, thus obviating the necessity for measurements in such areas. In this context, the location of a point (T_1) marked in the area close to the forest area border was determined by theoretical calculations, and the factors affecting the positioning accuracy were examined (Figure 1).

Initially, a suitable T_1 point was determined in the forest area where GNSS signals were found to be weak or completely blocked. In order to indirectly locate the T_1 point, two auxiliary points (1 and 2) were selected in the open area, with the aim of ensuring that precise measurements could be made with GNSS, and that the T_1 point could be seen directly. Subsequently, auxiliary points 3 and 4 were determined in the open area on the lines 1-T_1 and 2-T_1, respectively, thereby enabling more accurate calculations of T_1 coordinates.

Steps are followed to calculate the coordinates of point T_1 in the forested area.

•Based on the coordinates of points 1, 2, 3 and 4, the azimuth angles (1-3), (3-4) and (2-4) and the length "c" are calculated with the help of the second fundamental geodetic problem.

•Using the azimuth angles,

α = (2-4) - (4-3), β = (3-4) - (1-3) and γ= 200 - (α + β) are calculated.

•The lengths a and b are computed using the sine theorem ($\frac{a}{Sin(\alpha)} = \frac{b}{Sin(\beta)} = \frac{c}{Sin(\gamma)}$)

•The coordinates of point T_1 are calculated from points 3 and 4.

•Y_T = Y_3 + a*Sin (1-3) = Y_4 + b*Sin (2-4)

•X_T = X_3 + a*Cos (1-3) = X_4 + b*Cos (2-4)

As GNSS measurement could not be performed at the T_1 point, it was observed that the accuracy of the obtained coordinates, which were determined by theoretical calculations, depended on two factors. Firstly, the proximity of points 3 and 4 to the lines 1-T_1 and 2-T_1 was a determining factor, and secondly, the magnitude of the γ angle (Figure 2).

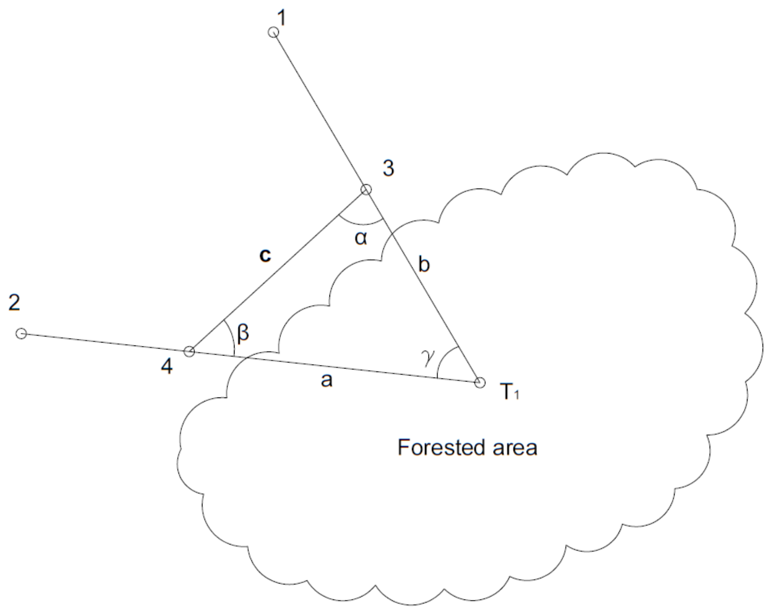

Figure 1: Network design for determining the location of point T_1

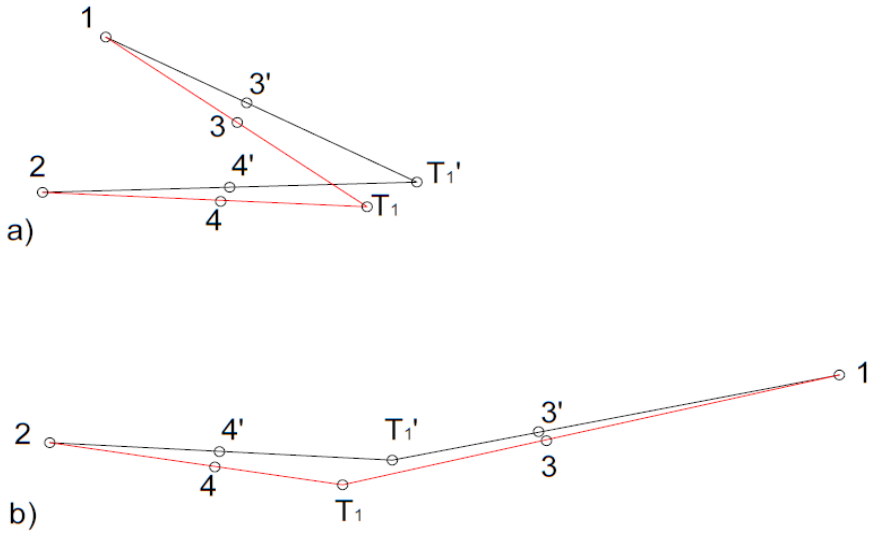

Figure 2: Plot of the change of position of the theoretical position T_1 and the realized pointT_1' around small (a) and 200g (b) values of the γ angle.

It was observed that when these points were positioned in close proximity to the measurement line, the calculated T_1 coordinates exhibited optimal accuracy, while as the deviations from the lines increased, the results exhibited a negative trend. Additionally, it was noted that if the γ angle was particularly small, approximately 200g (or around 180°), the calculated T_1 positioning accuracy underwent a decline. In the final stage, the T_1 coordinates were calculated theoretically, and the results were compared with different methods. Error analyses were also performed, with particular attention paid to the location of points 3 and 4 relative to the lines, the effect of different values of the γ angle, and comparisons with open area GNSS data.

These analyses were used to test the accuracy of the methods. The evaluation process concluded with the conclusion that this method, which is applied using auxiliary points in forest areas where GNSS signals are weakened or blocked, is a practical alternative that provides sufficient positioning accuracy.

Experiments

The experiment was conducted in two distinct wooded areas within the Davutpaşa Campus of Yıldız Technical University. This study investigates a practical solution that aims to overcome the positioning problem that occurs in forested/wooded areas close to open areas. In order to test the accuracy of this solution, measurements made at two different angle values (γ1, γ2) were examined, and the problems that may be encountered during the positioning process were evaluated. The tests were carried out in an area without difficult terrain conditions (within the university campus area). However, since forested areas are generally regions where the slope changes rapidly and have more difficult conditions, the validity of the proposed method in such environments is planned as a separate study subject in the future. In the selection of points, meticulous attention was paid to ensuring the presence of two distinct geometry test sites characterized by a small γ angle (see Figure 3) and an approximate mass of 100g (see Figure 4).

Initially, on 3 October 2023, points P1 and P2 were established in the vicinity of the primary test site, while points P5 and P6 were positioned in the proximity of the secondary test site. GNSS measurements were conducted in static measurement mode at these points, and coordinates were derived based on the measurements obtained over duration of two hours. The GNSS measurements were performed using four Topcon HiPer SR GNSS receivers. The technical specifications of the device utilized include a sensitivity of 3 mm + 0.5 ppm for horizontal accuracy and 5 mm + 0.5 ppm for vertical accuracy in rapid static measurement mode (L1 + L2). On 19 October 2023, the GEOMAX ZOOM 30 (2 mm + 2 ppm) total station was utilized to measure the horizontal and vertical angles and inclined distances of all points (including T_1 and T_2) in the study area.

The coordinates obtained from measurements made based on points in the region close to the study area were used as reference data in testing other measurements. Concurrently, RTK measurements were conducted utilizing the ISKI-CORS system, employing the PALA reference point, thereby acquiring the coordinates.

On 20 October 2023, a series of geodetic observations were conducted as part of a comprehensive research program. These observations included five-minute rapid static measurements, utilizing the ISKI-CORS system, which were performed in an open area. Concurrently, two-hour static measurements were conducted at the T_1 point in the primary test area. The purpose of these measurements was to assess the compatibility of the static GNSS measurement method by comparing it with the coordinates obtained through the conventional terrestrial measurement method.

Figure 3: Map of the first study area (small angle with γ ≈ 20g)

Figure 4: Map of the second test area with an angle value of approximately 100g (105g in the study).

For all GNSS measurements (static/rapid static), the data recording interval was set at 1 second and the elevation mask was utilized as 7.5°. The evaluation of static and rapid static GNSS data was conducted using Topcon Magnet Tools 8.1.0 commercial software, and precise positioning was achieved through the utilization of precision ephemeris and Final IGS products. The objective of this experiment was to compare the accuracy of different measurement techniques and to analyses methods to enhance positioning accuracy in forest areas. The experiments were conducted in selected forest areas in the Davutpaşa Campus region of Yıldız Technical University. Two different geometries were selected for this purpose, where the angle (ɣ) was small (Figure 3) and around 100g (Figure 4). Initially, on 3 October 2023, points P1 and P2 were established in proximity to test site 1, while P5 and P6 were established in proximity to test site 2. GNSS measurements were then performed on these points in static measurement mode for duration of 2 hours, after which their coordinates were calculated

The measurements were taken with four Topcon HiPer SR (Rapid-static (L1 + L2) H: 3 mm + 0.5 ppm and V: 5 mm + 0.5 ppm) GNSS receivers. On 19 October 2023, the horizontal angles, vertical angles and slant distances of all points within the designated study area (including T_1 and T_2 points within the wooded area) were measured using a GEOMAX ZOOM 30 (2 mm + 2 ppm) total.

RESULTS AND DISCUSSION

In this study, the PALA station of the ISKI-CORS network was evaluated as a reference for processing both static and rapid static measurements. The processing and adjustment of GNSS data were carried out with the Topcon Magnet Tools 8.1.0 commercial software. On the other hand, the coordinates obtained from the RTK measurements were recorded to the mobile receiver unit based on the PALA reference point of the ISKI-CORS network.

The coordinates obtained through the total station measurement method were selected as the reference, and it was determined that the coordinates obtained from each measurement method were close to the reference data with an accuracy of cm (see Tables 1 and 2).With the exception of the T_1 and T_2 points, all other points had different coordinate values despite having a clear field of view, and these coordinate differences are due to the accuracy of each measurement method and measurement device.

The obtained results demonstrate that the static measurement method yielded the most compatible results with reference data, and that the method with the largest differences was the CORS technique. In the context of forest studies, it is generally accepted that calculating coordinates to an accuracy of 10-15 cm is sufficient for forest measurement (Pirti and Kurtulgu, 2023). The differences between the coordinate data obtained from the reference method and the theoretically calculated point coordinates were found to be 15 cm for station T_1 and 10 cm for station T_2. While the error was calculated higher due to the small angle of the T_1 point in the first test area, better results were obtained at the T_2 point in the second test area, which was closer to the right angle.

Table 1: Differences of the coordinate values obtained by each measurement method from the reference coordinate values for the first test area.

Sts tions	Total station-CORS		Total station-Statik		Total station-Rapid static	
ID	Δy (m)	Δx (m)	Δy (m)	Δx (m)	Δy (m)	Δx (m)
1	0.026	-0.002	0.007	0.000	-0.004	0.007
2	0.017	-0.008	0.001	0.005	-0.001	0.015
3	0.027	-0.008	0.008	-0.006	-0.002	0.003
4	0.024	-0.012	0.008	-0.004	-0.002	0.000
T_1			0.025	0.018		

Table 2: Differences of the coordinate values obtained by each measurement method from the reference coordinate values for the second test area

Stations	Total station-CORS		Total station-Rapid static	
ID	Δy (m)	Δx (m)	Δy (m)	Δx (m)
5	0.016	-0.006	-0.002	0.005
6	0.011	-0.009	-0.001	0.009
7	0.015	-0.004	-0.001	0.002
8	0.018	0.006	-0.001	0.000

Table 3: The execution times of the measurement studies carried out in the first test area.

Name of The Work Done	**Time (s)**
Points establishment and direction aligned	15-20
CORS measurements	3-5
Static Measurements	240
Rapid Static Measurements	25-30
Terrestrial Measurements	45

Tables 3 and 4 present the times required for obtaining all measurements. The shortest times required for the location determination process, including the establishment of the point (fix operation in each method), are exhibited in the proposed method. Although the static measurement method provides similar results, the fact that the data evaluation phase, precise ephemeris and the final IGS product require more than 2 weeks demonstrates the effectiveness of the proposed method. The GNSS positioning accuracy varies according to tree leaf type, with the minimum position error being higher than expected (12.13 m). This indicates the effect of cover type on GNSS signal propagation (Feng et al. 2021). Given that the measurement accuracy used for forest studies is about 10-15 cm, it is evident that the proposed method is a practical, sufficiently accurate and faster method.

Table 4: The execution times of the measurement studies carried out in the second test area.

Name of The Work Done	**Time (s)**
Points establishment and direction aligned	15-20
CORS measurements	3-5
Static Measurements	120 (only P5 and P6 points)
Rapid Static Measurements	25-30
Terrestrial Measurements	45

More precise and accurate positioning is obtained from total station measurements. However, while the use of the GNSS RTK device requires one operator, other methods require at least two people (Lovrinčević et al., 2024). While the accuracy of RTK measurements decreases depending on the multipath effect of the measurement environment and the blocking of the signal, measurement times increase. Safrel et al. (2018) obtained coordinate data with a measurement time of approximately 40 minutes and an approach of over 10 m in their study. Pırtı and Kurtulgu, (2023) achieved 10-15 cm accuracy levels in the horizontal component in characterized forest areas. Measurements with Total Station can take several hours depending on the complexity of the work to be done and the desired accuracy level (Mihelič et al., 2022; Cho et al., 2023). However, in the current study, point positioning can be achieved with an accuracy of 10-15 cm in 20-25 minutes. When other studies are taken into account, it is clear that it is a very practical solution with more accurate point positioning in a shorter time. Brach and Zasada (2014) obtained coordinates with an accuracy of up to 25 cm depending on the antenna length in their study with an extended antenna.

CONCLUSIONS

The aim of this paper provides a comparison of different GNSS measurement techniques in terms of their accuracy and applicability in the forest areas. Firstly, the location of points T_1 and T_2 in forest environments is determined by using auxiliary points in open areas. Then, different measurement techniques and methods are tested, including static GNSS, rapid-static GNSS and CORS-based positioning. Finally, the results of these techniques are compared with the reference data obtained from total station measurements. The outcomes demonstrate that static GNSS measurements provide optimal accuracy, exhibiting the smallest coordinate differences compared to the reference data. Rapid-static measurements also yielded satisfactory results, substantiating their viability as a viable alternative in scenarios where time constraints exist. Conversely, CORS-based positioning exhibited the maximum coordinate deviations. All GNSS-based methods attain coordinate accuracy within a range of 10-15 cm, which falls within the acceptable limit for forest measurements.

The positional accuracy of the T_1 and T_2 points indicates that the geometrical configuration of auxiliary points plays a pivotal role in determining location accuracy. In the initial test area, where the γ angle is minimal, larger discrepancies were observed at point T_1 (approximately 15 cm). Conversely, in the subsequent test area, where the γ angle approaches a right angle, point T_2 exhibited enhanced accuracy (approximately 10 cm). This finding underscores the pivotal role of the placement and geometry of auxiliary points in determining the ultimate positioning accuracy. In all static GNSS measurements, the Topcon Magnet Tools 8.1.0 commercial software was processed using the precision ephemeris and final IGS products to enhance precision. A comparison of the results reveals that static GNSS measurements exhibit superior precision, while the rapid-static technique provides a balanced trade-off between accuracy and efficiency.

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FACTORS AFFECTING FATTY-ACID PROFILE OF LACTIC LIPIDS IN THE WATER BUFFALO (*Bubalus bubalis*) – A REVIEW

SUMMARY

The aim of this review was to study how lactic fatty acids (FA) are affected by different factors in the different pathways in the buffalo – a species differing from cattle with its response to feeding strategies, metabolism and specific functional composition of milk. It underlines the role of management to manipulate enzymatic desaturation and bacterial synthesis and that of ripening and pasteurization in dairy technology. The review presents some proofs of effect of season on FAs, but it should be correctly discriminated from the effect of lactation stage, showing improved unsaturation in advanced lactation. Farming system has major impact, and it can include feeding strategies (rumen-inert fats, seeds, bioactive compounds, etc.) to affect rumen biohydrogenation, but it is implied that the doze is important. In fact, the best control over bubaline lactic FAs is via natural grazing, improving omega ratio, conjugated linolenic (CLA) and *trans*-vaccenic acid (TVA). This is not only because of plants' composition of soluble sugars, vitamins, polyphenols and proteins, but mostly because of the stimulated bacterial synthesis and Δ9-desaturase activity, responsible for the *de novo* synthesis. The results about the transformations in the beneficial fatty acids in the yoghurt production are controversial explaining the predominantly negative impact mostly with pasteurization. Cheese processing generally alters the individual isomers but not the groups of beneficial fatty acids (CLA) as a whole chiefly due to ripening and pasteurization (only if the temperature is high), but not due to renneting.

Key words: *Bubalus bubalis*, milk, fatty acids, grazing, supplementation, dairy processing

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INTRODUCTION

Buffalo milk is a delicacy product characterized by high density – associated not only with its higher dry matter, but also with the high proportion of high-melting triglycerides (Ramamurthy and Narayanan, 1971; Khan, T.I. et al., 2019) – and by specific, appealing odor, attributed to some volatile organic compounds (Moio et al.,1993)and to the high concentrations of short-chain (SCFA) fatty acids (Naydenova, 2005; Güler et al., 2005). In buffalo milk fat content is double higher but cholesterol is lower, and the lipid globules are larger, as compared to cow milk (Zicarelli, 2004; Islam et al., 2014). Noteworthy is also the presence of gangliosides, which are not found in cow milk and which have anti-inflammatory and anti-toxin effects (Ahmad et al., 2013).

Buffalo milk is rich in whey proteins, calcium and in particular colloidal Ca (Nguyen et al., 2014; Islam et al., 2014). According to Chandan et al. (2006), during dairy processing all these components enhance the effect of probiotic bacteria, which inhibit the absorption of cholesterol and hence significantly reduce blood pressure.

Bulgarian yoghurt (germinated with *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*) is a specific type of fermented dairy product that has gained recognition on the world market. Such probiotic microflora has synergistic effects that result in specific texture, composition and sensory properties of that dairy product (Ebringer et al., 2008). Buffalo yoghurt is characterized by microstructure that causes higher syneresis – interrupted by large fat globules, binding less protein and featuring more serum pores (Nguyen et al., 2014; Abesinghe et al., 2020), as well as by higher titratable acidity associated with firmer and smoother coagulum (Naydenova, 2005). Both yoghurt and cheese are natural carriers of probiotics with essential effects on the immune system and gastrointestinal health (Abesinghe et al., 2020).

Casein in buffalo milk is roughly 80% of total protein (20% whey protein) and almost all of it is in the form of larger more numerous micels, as compared to bovine milk (Ahmad et al., 2013). The particular role of high k-casein and superb proportions amongst protein, whey protein, types of casein and fats are important for the outstanding rennet coagulation, gelling and firming properties in the production of curd (Ariota et al., 2007; Abesinghe et al., 2020; Islam et al., 2014).

Moreover, the higher fat content of buffalo milk is essential as it leads to higher ratio with protein, also contributing to the superior elastic property of the curd (Ariota et al., 2007). In association with the higher fat content and dry matter, during ripening buffalo cheese is characterized by lower intensity of physical and chemical changes and lower lipolytic activity, as concluded by Ivanov et al. (2016).

Compared to cow, buffalo milk is characterized by higher saturated fatty acids, mainly palmitic acid, *trans* fatty acids, and conjugated linolenic acid (Ménard, et al., 2010; Penchev et al., 2016).

The essential nutritional values of milk and dairy products are indisputable but still there is criticism against their mass consumption, because of the saturated nature of the lactic lipids (Givens and Shingfield, 2006). But, as ruminant products, they are a dominant provider of the beneficial conjugated linoleic acid (CLA), trans-vaccenic acid (TVA) and other monounsaturated (MUFA) acids, and even some saturated fatty acids (SFA) like butyric and stearic (German, 1999; Lawson et al., 2001; Vargas-Bello-Pérez and Garnsworthy, 2013). Buffalo milk was also established to reduce cancer risks in a trial of Ramirez et al. (2013). Thus, milk and its derivative foodstuffs have been proved to have anti-cardiovascular, anti-mineral fertilizing was applied. carcinogenic, anti-atherogenic, anti-obesity, anti-diabetic effects and stimulate immune system (Belury, 2002; Parodi, 2004; Dilzer and Park, 2012).

In addition, fatty acids are indicative of the quality of the different types of cheese, as they are strongly responsible for the formation of flavor and they are precursors for other volatile aromatic compounds (Khalid and Marth, 1996).

There are two main sources of fatty acids in milk – originally formed, unchanged FAs and such synthesized *de novo* in the mammary gland (Chilliard et al., 2000). The *de novo* FAs are synthesized from acetate, butyrate, and volatile FA which are a product of fermentation of cellulose and hemicellulose by specific bacteria in the rumen. In the mammary gland, the acetate and β-hydroxybutyrate are precursors of the fatty acids with 4 to 12 carbon atoms and great parts of the myristic acid and palmitic acids (Shingfield et al., 2013). The rest part of the latter two FAs originates from circulating lipoproteins rich in triacylglycerols and also from intestinally absorbed lipids and such from body fat mobilization (Bauman and Griinari, 2003; Shingfield et al., 2010).

Long-chain fatty acids (LCFA) are some of the FAs deriving from elongation of diet FAs or such deposited in the udder, and in particular long-chain saturated fatty acids (LCSFA) like C20:0, C22:0 and C24:0 may derive both from diet and body fat mobilization (Correddu et al., 2017). Odd- and branched-chain (BCFA) and other valuable FAs come via completely different pathway. Though rumen microorganisms apply biohydrogenation to polyunsaturated fatty acids (PUFA) which converts them to SFA (Jenkins et al., 2008), in this process some bacterial genera produce wide range of intermediates with high benefits, like rumenic acid (Palmquist et al., 2005).

An important feature of milk and dairy products is how their fatty-acid profile is affected by environmental, physiological and especially managemental factors and how it can be manipulated through the different pathways to produce more beneficial foodstuffs. There are plethora of studies and experiments on dairy cows, but such regarding animal effects, environmental and managemental factors, rumen-inert supplementation, and dairy processing specifications on the fatty acids of buffalo lactic lipids (in milk and dairy products) are limited. Because, though appearing similar, the body response to the complex synthesis of these metabolites in the two species should be considered with different respect, as suggested in their response to roughage shift in our previous study (Penchev et al., 2016) and to the metabolic pressure during the transition period (Pegolo et al., 2017).

MATERIAL AND METHODS

A profound comprehensive search of scientific literature was conducted in order to study the problem in focus. In the present review information from publications obtained on the Internet – exclusively Google Scholar but mostly Research Gate, PubMed, Scopus and Web of Science – as well as journal editions worldwide and also editions in Bulgaria were used.

More specifically, the expertise and research experience in fatty-acid analysis of buffalo production and the experimental results of our animal breeding team with regard to diet supplementation were also taken in consideration as a foundation for this literature review.

RESULTS AND DISCUSSION

The current technology for fatty-acid analysis of milk allows identification of a great number of molecules from the groups of BCFA, *cis* and *trans* isomers of 18:1, 18:2 and 18:3, which are involved in the processes in the rumen, *de novo* synthesized in the mammary gland, and other FA deriving from elongation of diet FA or such deposited in the udder, including LCFA (Correddu et al., 2017).

Wide range of fatty acids, including low-concentration FAs precious for the consumer, was analyzed by Pegolo et al. (2017) for the milk of Italian Mediterranean buffaloes from six farms using one and the same ration from January to May (from cold to warm season). Lactation phase was found to have significant effect. The dynamics show that after increase in early lactation, the medium-chain fatty acids (MCFA) decrease generally throughout the period, while for LCFA it is the opposite – a general increase after initial decrease. MUFA were also found to increase generally, also after an initial decrease until day 120. These changes in the trend of the dynamics of the lactic fatty acids from fourth month on might be associated with the end of the period of negative energy balance, though in this species it is slightly expressed (Pegolo et al., 2017), or with overcoming the period of highest productivity, the peak being established usually in the fifth week postpartum in the Italian buffaloes (Borghese et al., 2013). Total PUFA were also found to increase generally after an initial decrease until day 120, and the increase in the rumenic acid in particular was straightforward.

The observations of these Italian authors about PUFA (on as many as 272 heads) are supported by the results of Zotos and Bampidis (2014) who monitored the fatty-acid changes in smaller number of dairy Greek buffaloes (also Mediterranean type, n= 40) from September to February (from warm to cold season – opposite to Pegolo et al., 2017), finding an increase in the concentrations of the beneficial PUFA and CLA. Similar dynamics in PUFA with the advance of lactation in dairy buffaloes was observed also by Sharma et al. (2000), while Verdurico et al. (2012) did not find any significant changes as it was in our study on the Bulgarian Murrah, especially in buffaloes under intensive farming (Ilieva et al., 2020). On the other hand, Zotos and Bampidis (2014) did not establish significant alterations in butyric, vaccenic and rumenic acid which is different from the Italian study.

There are two sources of fatty acids in milk. Except for the circulation of preformed FA, there is also *de novo* synthesis in the wall of the mammary gland

(Chilliard et al., 2000). One such FA synthesized *de novo* is the butyric acid with the mediation of acetyl-CoA carboxylase and FA synthase enzymes (Chilliard et al., 2000), and another is the *trans*-vaccenic acid (C18:1*trans*-9) and it depends on the activity of Δ9-desaturase (stearoyl-CoA desaturase), being a catalysator of the desaturation of C18:0. In a study to characterize the fatty acid profile of buffalo milk, Correddu et al. (2017) pointed out that desaturation is involved in the production of most of the *cis* and *trans* isomers of C18:1 and CLA *cis*-9,*trans*-11.

In correspondence with the study of Pegolo et al. (2017) about PUFA, the buffaloes from the Mediterranean breed in Brazil were also found to slightly but steadily increase this value in their milk throughout lactation (Verdurico et al., 2012), and the Murrah breed in India to have highest PUFA in the most advanced stage of lactation (Sharma et al., 2000).

Nevertheless, unlike the above cited studies, Qureshi et al. (2012) came to the conclusion that the higher lipid value of milk is in early lactation stage.

While in the above quoted studies variability of fatty acids is attributed to the animal factor lactation stage, the research of Talpur et al. (2008) provides evidence of the actual impact of the environmental factor season of test day in buffaloes in Pakistan, as well as in other ruminants. This was associated with the availability of fresh grass during summer and hence with the better biohydrogenation. Substantial difference was found in CLA, showing higher summer level by 33% compared to winter, and especially in rumenic acid in particular – by 72%. Respectively, total PUFA (by 15.7%) and in particular 18:3n-3 (by 58%) were also higher in summer season; MUFA and TVA – by 12 and 42% respectively. Total saturation of buffalo milk dropped from winter to summer by 7%, including the decrease in C18:0 by 22%. All this was confirmed in the dairy cows, ewes and goats within the same study.

In contrast, Nie et al. (2022) did not find seasonal alterations in Chinese crossbred buffaloes in the main FA groups (SFA, MUFA, PUFA) and also in butyric, TVA and rumenic acids. Nevertheless, the concentrations of some SFAs (C8:0, C10:0, C18:0) were significantly lower in summer milk, while other SFAs (C15:0, C15:1, C16:0, C17:0, C17:1, C15:0) were higher; C18:3(ω-6), C18:3(ω-3), C20:0, C20:2, and C20:4 were also higher. More importantly, in agreement with the previous citation, total PUFAn-3 were significantly higher in summer, and the ratio n-6/n-3 was lower; atherogenicity and thrombogenicity indices were also favorable in the summer. SCFA were lower in summer, while MCFA and LCFA did not vary between seasons.

In total disagreement, for buffaloes in the conditions of India Saroj et al. (2017) established opposite tendency – all the FAs valuable for health were higher in the winter season. Though the ration of the studied dairy animals was principally the same in the two seasons, the source of green roughage was different, transferring the “resposibility” for the changes upon the factor feeding/management. Similarly, in Iranian buffaloes, Chashnidel et al. (2007) found higher PUFA and CLA in summer due to forage resources.

Controversial are also the results of Naydenova (2005), finding higher SFA in the buffalo milk produced in spring/summer for one farm and the opposite for another. The same concerns in particular the *de novo* synthesized SFAs with 6 to

12 atoms, and also those of mixed origin from C14:0 to C16:0. This author also shares the opinion that all changes are associated mostly with forage resources than with seasonality of FA synthesis, hydrogenation, microflora development, etc.

Buffalo milk produced in summer should be considered more beneficial than that in winter not only because of the effect of season and the related forage resources as associated with the high activity of Δ9-desaturase and acetyl-CoA carboxylase rather than to the actual component composition of the diet, namely the botanical properties of the plants.

As Pegolo et al. (2017) also found, parity did not have significant effect on most of the fatty acids, but it affected BCFA and also MUFA, in particular oleic acid (n-9), which is the Δ9-desaturase major product. The study of Qureshi et al. (2012), though, established effect of parity on the concentrations of C14:1, C16:1 and C18:3, showing higher values in younger buffaloes. In similarity with Pegolo et al. (2017), C18:1*cis* was also affected, together with MCFA and LCFA which were found in higher concentrations in older buffaloes. The Δ9-desaturase activity was significantly higher in younger animals, but it was not affected by lactation phase. To support this, colostrum exhibited lower levels of saturation and n-6/n-3 ratio compared to mature milk (Zhigao et al., 2024).

Effect of parity was observed also in other studies on bovine (Nogalski et al., 2012; Samková et al., 2012) and bubaline cows (Qureshi et al., 2012), mainly based on the emphasized effect of the earlier months postpartum in lactating cattle (Stoop et al., 2009; Wang et al., 2013) and buffaloes (Sharma et al. 2000; Verdurico et al., 2012).

Qureshi et al. (2010) reported that with the increase of body condition score (BCS) in dairy Nili-Ravi buffaloes the SCFA/MCFA ratio increased, which applies also to the concentration of C18:0. Total unsaturated fatty acids (UFA) and C18:2*cis*-9, -12 and C18:3*cis*-9, -12, -15 in particular, and also C18:1*cis*-9 have highest concentrations in the lactating animals with moderate BCS.

As implied above, in its broader sense, the effect of season in most cases suggest an effect of feeding and farming system. Ilieva et al. (2020) found substantial beneficial effect of pasture on all important fatty acids in milk – especially in the *trans*-FA, like rumenic and vaccenic, omega-6/omega-3 ratio, total MUFA and PUFA, and to a smaller extent in atherogenicity and thrombogenicity index. In the same time, there was a decrease of the levels of some FAs (TVA, GLA, CLA, total PUFA) in milk with the advance of the pasture season which is to be attributed to the botanical and phenological changes of the grassland (Ferlay et al., 2008; Gorlier et al., 2012) and its protein content (Elgersma et al., 2003).

In two other grazing herds of Bulgarian Murrah, the results of Naydenova (2005) showed that the most atherogenic C14:0 and the C12:0 are higher but C16:0 is lower, MUFA is higher and PUFA is definitely lower in comparation to that study of Ilieva et al. (2020). In Colombia (Bustamante et al., 2017) and Brazil (Gagliostro et al., 2015), the beneficial FAs in milk from pasture were generally higher, and in Romania (Vidu et al., 2015) – lower.

The effect of grazing is associated with stimulation of Δ9-desaturase (Shingfield et al., 2005; Kalač and Samková, 2010), responsible for the synthesisis of *trans* isomers (Bauman et al. 2006) by adding a *cis*-9-double bond on the FA chain (Shingfield et al., 2008). On one hand, 25% of TVA (*trans*-11-18:1) that is synthesized in the rumen is *trans*formed by mammary Δ9d to rumenic acid (*cis*-9,*trans*-11-18:2) (Mosley et al., 2006), so being responsible for more than 70% of that CLA in milk (Shingfield et al., 2013). On the other hand, pasture farming has an impact on ruminal biohydrogenation of unsaturated fatty acids (UFA) and on the growth of specific bacteria in the rumen on the basis of higher concentrations of soluble sugars in fresh plants compared with conserved roughage (Kelly et al., 1998; French et al., 2000). This stimulates Δ9d, respectively CLA, via mammary pathway and prevents the reduction of vaccenic to stearic acid (Nudda et al., 2005), and also enhances synthesis of PUFAn-3 (Chilliard et al., 2001; Dewhurst et al., 2006) via rumen bacteriome. In buffalo, mammary Δ9-desaturase enzyme activity was established greater in pasture farming explaining the higher CLA (Fernandes et al. 2007).

In these processes, it has an important role especially in the desaturation of C18:0 to TVA (Ntambi & Miyazaki, 2004; Bernard et al., 2013), with established higher activity in grazing buffaloes as compared to such bred intensively (Fernandes et al., 2007).

In this way, the degree of biohydrogenation can be judged by the level of stearic acid, as Nielsen et al. (2006) suggest. The tendency for diminishing biohydrogenation as a result of lower pH (Talpur, 2008) in response to silage feeding (Jalč et al., 2013), is confirmed in the buffaloes from the study of Penchev et al. (2016) where MUFA, PUFA, linoleic acid and n-6/n-3 ratio tended to improve with the consumption of alfalfa hay after replacing silage in the diet. This change in the roughage source presumably appears as a stress factor resembling the phenomenon of negative energy balance, regardless of the lactation stage causing mobilization of LCFA from body fat reserves. To our personal practical knowledge, Bulgarian Murrah buffaloes would the sooner compromise their milk production than change their lipid metabolism, which explains the steady pattern of alteration of FA profile of milk during the period of roughage shift. In addition, as a species, the buffalo is also rather unpretentious to forage and such diet shift would not lead to any major misbalance.

In this context, organic buffalo milk and mozzarella showed significantly higher CLA*cis*-9,*trans*-11, TVA and antioxidants than conventional production in a study of Bergamo et al. (2003) in the Italian Mediterranean breed. Similarly, Uzun et al. (2018) found higher PUFA in lactating buffaloes from the same breed fed fresh sorghum roughage, which was preserved in the mozzarella dairy processing.

The fatty-acid profile of most forages, feeds (including some oilseeds), and some by-products used in ruminants' feeding contain UFA with 18 carbon atoms, predominantly C18:2*cis*-9, -12 and C18:3*cis*-9, -12, and -15. Some oilseed supplements have high levels of MUFA, especially C18:1*cis*-9 (Morales and Ungerfeld, 2015; Dewanckele et al., 2020). The best examples for biohydrogenation is the isomerization of 18:2n-6 to CLA*cis*-9,*trans*-11, then hydrogenated to TVA (18:1*trans*-11) and finally to stearic acid (18:0) (Bauman & Griinari, 2003).

The scientific literature shows that ruminal bacteria proliferation and activity can be improved by nutritional manipulation reducing UFA biohydrogenation which can affect milk fatty-acid quality. The effects of supplementing diet with rumen-inert fats on the productivity are well presented for dairy cows, but such reports for the buffalo are limited, especially with regard to fatty acids. The enzymatic activity of Δ9-desaturase is definitely affected by the lipid content of the diet (Ntambi and Miyazaki, 2004; Bernard et al., 2013).

Varricchio et al. (2007) observed that protected fats (calcium salts of fatty acids) did not have positive results in the subjected Mediterranean lactating buffaloes, increasing the level of C16:0. Nevertheless, the supplemented whole cottonseeds from the same publication had beneficial effect on the fatty acid profile, namely n-6/n-3 ratio, PUFA, atherogenicity index and CLA. In Murrah buffaloes in India, Shelke et al. (2012) also found that supplementing rumen protected fat did have effect on milk fatty-acid profile, expressed in a 36% increase in UFA.

In their 21-day experiment on 12 adult Nili Ravi buffaloes, Hifzulrahman et al. (2019) tested the effects of supplementation of rapeseed oil, calcium salts of palm oil and high palmitic acid on dry matter intake, milk yield, milk composition and fatty-acid profile. The authors found increased MCFA in the calcium and palmitic groups, while rapeseed supplementation increased LCFA.

Santillo et al. (2016) observed that buffalo supplemented higher level of flaxseed (1000 g per capita per day) had lowest concentration of SCFA, in particular C8:0 and C10:0, compared to lower flaxseed diet (500 g/d) and the protein diets. In the group with 1000 g/d flaxseed MCFA were also lowest, in that with 500 g/d – intermediate, and in the high and low protein groups the concentration was highest. In LCFA it was the opposite – highest in high supplementation, intermediate in low flaxseed, and lowest in milk produced after no flaxseed in the diet of the buffaloes. Total CLA followed the same trend as LCFA, higher by 22% in the 1000 g/d group compared to the control. Atherogenicity and thrombogenicity index and n-6/n-3 ratio were most beneficial when the flaxseed was highly presented in the diet. Protein level of the diet showed no specific effect on the fatty acids in milk, especially in combination with flaxseed supplementation, the sole effect of protein being significant on C18:0 and C18:1*trans*-11 only. The Δ9-desaturation was found to be with higher activity after feeding low-protein diet and was not affected by flaxseed supplementation.

The authors (Santillo et al., 2016) also pointed out that this supplementation might not have the same effect in different ruminant species and even different breeds of buffaloes in view of the different gene expression and hence different activity of Δ9-desaturase, on the basis of its high genetic variability observed in the bubaline species (Pauciullo et al., 2010).

In contrast to these authors' conclusions about protein, Tyagi et al. (2007) found that feeding the high-protein berseem to buffaloes can serve as a natural diet manipulation, resulting in milk with more favorable concentrations of total CLA, rumenic acid, PUFAn-3 and n-6/n-3 ratio which was established to be preserved in the processing to three types of cheese.

Patiño et al. (2012) established that the diet supplementation of 140 ml per capita per day of fish oil resulted in constant PUFA n-6 and improved concentrations of CLA and PUFA n-3 in buffalo milk, while using lower doze of 70 ml had positive effect only on the latter.

Hassan et al. (2021) established no effects of supplementation of mixture of black pepper fruit, ginger, cinnamon, peppermint, ajwain seeds and garlic on milk performance, and more importantly on ruminal fermentation, and bacterial diversity in Murrah buffaloes in China. Nevertheless, they observed significant increase in the

in Murrah buffaloes in China. Nevertheless, they observed significant increase in the concentrations of PUFA when a lower doze of 20 g per capita per day was used. A significant decrease of SFA was found as a result and also an increase of MUFAs like C14:1, C16:1, C18:3 and C18:2n-6.

As Vlaeminck et al. (2006) points out, the latter acid is found highly presented in concentrate feeds. Ruminants with high biohydrogenation have highly presented products of C18:2n–6 in milk which is associated with intensive farming, with large use of concentrates and supplements. According to the authors, the effect of diet is associated with ruminal microorganisms, namely their growth and activity dependent on forage type, forage-to-concentrate ratio, supplementation, secondary plant metabolites and bioactive compounds. Such feeding strategies affect BCFA in milk, reported also for the buffalo in particular (Correddu et al., 2017). BCFA are mainly derivative product of bacteria that leave the rumen and are responsible for fluidity of cell membrane of rumen bacteria and their concentration in milk can be used as a diagnostic indicator of the microbial variety and abundance in the rumen (Vlaeminck et al., 2006).

The SFAs with 4 to 12 carbon atoms are synthesized by mammary gland from acetate. Great portion of myristic and palmitic acid in milk fat come through the same pathway (Shingfield et al., 2013) while the rest part of them originates from circulating lipoproteins rich in triacylglycerols and also from intestinally absorbed lipids and such from body fat mobilization (Bauman and Griinari, 2003; Shingfield et al., 2010). Odd-chain fatty acids (OCFA), BCFA and other valuable FAs come via completely different pathway.

Though rumen microorganisms apply biohydrogenation to PUFA and converts them to SFA (Jenkins et al., 2008), in this process some bacterial genera produce wide range of intermediates, like rumenic acid (Palmquist et al., 2005). Another finding of Hassan et al. (2021) was that the herbal preparation stimulated the rumen bacteria of the genera *Succinivibrionaceae*, *Butyrivibrio*, *Pseudobutyrivibrio*, and *Lachnospiraceae* improving fatty-acid profile of milk. Positive correlation was established between C18:3n-3 in milk and specific groups of bacteria showing a tendency to increase PUFA in buffalo milk.

The main source of energy for animals are volatile fatty acids (Mizrahi, 2012) which are produced by rumen bacteria via degradation of polysaccharides and cellulose (Flint et al., 2008). Other precursors of *de novo* synthesized FAs are acetate and butyrate. Buccioni et al. (2015) reported about the capacity of polyphenols to improve the activity of Δ9-desaturase enzyme (SCD), taking part in the conversion of stearic to oleic acid and TVA to CLA, especially increasing n-3 and n-6 fatty acids at a par with rumen biohydrogenation, as well as α-linoleic acid (Roy et al., 2002; Cabiddu et al., 2010). Such limited data about bioactive compounds on lactic fatty acids were revealed in our previous, still unpublished review, which found no reports on buffalo milk.

The results of our experiment on Bulgarian buffaloes (Ilieva et al., 2022) indicate that the content of MCFA, such as lauric and capric acids, decreased while the concentrations of LCFA (11-hexadecanoic and stearic), increased in the group of buffaloes supplemented AyuFertin, containing bioactive compounds such as carotenoids, flavonoids, tocopherols, and fatty acids. In another experiment of our team (Penchev et al., 2022) was concluded that, in comparison to the control group, in the milk from the buffaloes consuming curcumin total and individual SFA have not changed, which applies also to the produced yoghurt. There was increased concentration of the valuable TVA and total *trans*-isomers in the raw milk product of supplementation, which is obliterated after processing to yoghurt. The dominating MUFA, oleic acid, was found constant.

Studies also demonstrate that for the significant individual variation in unsaturation of the milk fats also genetics plays a role, Kelsey et al. (2003) and Soyeurt et al. (2006) having even established that in cows the within-breed variation is greater than between breeds. Similarly, in national cattle Mihaylova and Peeva (2007) has found greater variation among regions than among breeds, supported by Dimitrov (2007) for the Bulgarian Black and White breed. In the Bulgaian Murrah buffalo the author (Mihaylova and Peeva, 2007) observed general uniformity among herds, with greater variability of 14- to 18-atom FAs (especially of the most highly presented palmitic acid) in our herd as compared to three other herds.

In the processing from milk to dairy products – namely white brine cheese, Bulgarian yoghurt and curd – in our study (Ivanova S. et al., 2021) in Bulgarian Murrah buffaloes myristic acid was found to change to little extent. As compared to milk, in the yoghurt from two studies of Naydenova (Naydenova, 2005; Naydenova et al., 2013) were observed similar changes in the 4- to 14-carbon FAs – especially the increase of capric, lauric and myristic acids. But the increased content of the palmitic acid and the decreased stearic and oleic acids are in disagreement with the unchangeable values found by Ivanova S. et al. (2021). In agreement between the three studies, total dienes were established to decrease in yoghurt, while Naydenova (2005) and Naydenova et al. (2013) observed an increase in the trienes and decrease in total MUFA which is a difference. In the same time, using technological methods to elongation of shelf life in yoghurt from buffalo milk, Ivanova S. et al. (2021) have found unchangeable SFA.

Ivanova S. et al. (2021) also established no specific changes in MUFA (TVA in particular) and *trans* isomers in the studied processing to the three dairy products of buffalo milk.

As for the saturation of the cheese, the lauric, caprylic and capric acids that showed some increase in the cheese making process, except for its adverse atherogenic effect, have also some benefits in association with antivirus and antibacterial effects (Sun et al., 2002; Thormar and Hilmarsson, 2007), the latter two together with butyric acid forming the specific flavor of cheese (Naydenova, 2005; Güler et al., 2005).

More special is the increase of C4:0 as it is found only in fats of ruminant origin and which, together with the fat-soluble vitamins (A, D, E) and CLA, has protective function against various diseases (German, 1999; Parodi, 2004). In disagreement, in buffalo un-ripened cheese, Van Nieuwenhove et al. (2007) established increased rumenic, total CLA, stearic acid, and ∑C18:1*cis*. Similarly, Tyagi et al. (2007) found in the production of three types of cheese that the improved total CLA, rumenic acid, PUFAn-3 (respectively n-6/n-3 ratio) after feeding the high-protein berseem to buffaloes were preserved in the end dairy products.

Similar significant increase in the concentration of butyric acid in the dairy processing to the fermented buffalo milk Dahi was found by Yadav et al. (2007), when using germination wih *L. acidophilus* and *L. casei*. Moreover, the levels of CLA were twice higher and the storage at 4°C preserved all CLA isomers.

Santos-Junior et al. (2012) found rumenic acid to undergo dramatic drop due to pasteurization and to partially regain its content during fermentation. The author' conclusion that the drastic decrease in CLA obliterates the efforts to increase it via diet supplementation explains the findings of our study (Ivanova S. et al., 2021) but is not commensurate with the following review.

Gutiérrez (2016) finds the literature on the effect of fermentation during dairy processing rather controversial and concludes that the content of CLA in dairy foodstuffs depends more on feeding and management than on the specifications of the milk processing. According to Dave et al. (2002), the concentrations of CLA, TVA and PUFAn-3 remain unchanged in yoghurt produced via germination with *L. d. bulgaricus* and *S. thermophilus*, although the diet manipulation of fatty-acid profile resulted in substantial increase of beneficial FAs in milk. But still, there are evidences that these two probiotic strains efficiently biosynthesize CLA from linoleic acid (Lin et al., 1999; Yang et al., 2014).

In the study of Ivanova S. et al. (2021) the thermal regime of the cheese making is not very high, for which our practical observations in laboratory conditions have shown that it does not affect fatty acids, so the alterations in dairy processing are to be associated with the further processes – to lesser extents with fermentation and to greater with ripening and aging. The different behavior of CLA can be explained with the difference in pasteurization specifications. Martínez-Monteagudo and Saldaña (2014) established that both CLA and TVA are oxidized to a great extent when the temperature increased from 90 to 120°C.

A process of oxidation was suggested to happen to the *trans*-vaccenic acid in the yoghurt production (Ivanova S. et al., 2021), meaning that a further increase must have also occurred presumably due to biosynthesis. This is for compensation of the otherwise serious reduction of total CLA, as TVA is reported to be the only known precursor of rumenic acid and a definite portion of it from the human diet is converted into it (Turpeinen et al., 2002; Field et al., 2009).

Except thermal specifications of the processing of milk, according to Khan, I.T. et al. (2020) cooling after coagulation also plays effect which is expressed in decreased concentrations of MUFA (C18:1) and PUFA (C18:2) in yoghurt cooled down to 25 and 18ºC but when it is cooled from 43ºC way down to 5ºC they retain their levels.

Like in the yoghurt, in the curd produced via acidification of Ivanova S et al. (2021) was established dramatic decrease in total CLA and in all individual CLAs, excluding rumenic acid, as it had been in a similarly produced but germinated ricotta cheese during 6-month ripening (Bergamaschi and Bittante, 2017).

CONCLUSIONS

As indicated by their reaction to the change in roughage in our earlier study and the metabolic variations mostly during the transition phase, the buffalo's body response to the complex synthesis of fatty acids should be distinguished from that of dairy cattle. One specific feature of this species is its seasonality (at least with regard to reproduction), so respective dynamics of the valuable FAs can be expected, as some studies have shown, but diet changes should be carefully weighed to avoid masking of the *per se* factor. The most mitigative effect is the animal factor lactation stage, expressed in improvement of beneficial FAs with the advance of lactation. But the highest effect is associated with managemental practices.

Though, as a ruminant product, buffalo milk acquires only limited number of FAs directly from the diet, farming system involving feeding plays the major impact on the composition of fatty acids in it. It can also include fatty-acid manipulation via different feeding strategies (rumen-inert fats, seeds, bioactive compounds, etc.) to affect rumen biohydrogenation but the inconsistent success in this implies that the doze should be studied very well.

The best manipulation, as many studies show, is natural grazing, not only because it provides soluble sugars, vitamins, polyphenols and proteins, or even some FAs that enter unchanged the bloodstream, but mostly because of the stimulation of the microbial synthesis, and the activity Δ9-desaturase and acetyl-CoA carboxylase responsible for the *de novo* synthesis, in these pathways improving omega ratio, conjugated linolenic (CLA) and *trans*-vaccenic acid (TVA). The results about the transformations in the health-related fatty acids in the yoghurt production are controversial, while those about the cheese confirm to some extent the general conception that such technological processes alter the individual isomers but not the groups of beneficial fatty acids (CLA) as a whole.

The resemblance in the changes between yoghurt and curd (with no germination) presumptively denies the existence of a notable effect of fermentation and emphasizes the negative effect of pasteurization. In cheese making, to these effects should be added the impact of ripening processes and that of pasteurization only if the temperature applied is high, but not the effect of rennet, which has no lipolytic activity.

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Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

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The International System of Units (SI) should be used.

- CONCLUSIONS

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- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

- REFERENCES (LITERATURE)

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