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ORIGINAL ARTICLE

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ENDEMIC FLORA WITHIN COMMUNITIES OF MOUNTAIN PINE (*Pinus mugo* Turra) AT METOHIAN PROKLETIJE MOUNTAIN

ABSTRACT: Metohian Prokletije is marked as one of the main European centers of the Balkan floristic endemism due to their geographical position on the fringe of the Mediterranean Sea and complex and diverse geomorphological, geological, orographic and climatic factors. This paper presents the results of long-standing comprehensive floristic researches of endemic flora in the Metohian Prokletije Mt., with particular reference to their high mountainous areas over the tree limit where *Pinus mugo* communities are concentrated. These types of ecosystems represent a transitional zone between the coniferous forests and high mountain bushy and herbaceous vegetation, at an altitude of 1,700 m do 2,400 m. Based on detailed field surveys, 118 Balkan endemic plant taxa within these communities were recorded so far. The phytogeographical analysis showed that the Balkan endemic taxa predominantly belong to the South-European mountainous (SEM) and Central-European mountainous (CEM) chorological groups with the following participation of floristic elements: 1) Dinaric-Scardo-Pindhian-Moesian (44 taxa or 37%), 2) Dinaric-Scardo-Pindhian (40 taxa or 34%), 3) Dinaric (24 taxa or 20%), and 4) Dinaric-Moesian elements (10 taxa or 9%). The biological spectrum was characterized by the prevalence of hemicryptophytes and chamaephytes. The obtained results on the presence of numerous endemic plants in the studied area can increase the interest in further floristic research, while the provided information of the ecological importance as well conservation status of threatened plant communities with *Pinus mugo* aimed at encouraging more effective biodiversity protection and sustainable management of natural resources.

KEYWORDS: Endemic flora, communities of mountain pine (*Pinus mugo*), Metohian Prokletije

INTRODUCTION

The Prokletije Mountains are located in the extreme southeast of the Dinaric region within the wider Mediterranean mountain chain zone. They are one of the highest and most rugged mountain ranges on the Balkan Peninsula situated in the border zone of southern Serbia (Kosovo and Metohija), eastern

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Montenegro and northern Albania. The northeastern part of the Prokletije Mountains belongs to the Metohian Prokletije.

The geographical position on the fringe of the Mediterranean Sea (Adriatic Sea), complex and heterogenous geomorphological properties of terrain and geological composition, and various climatic conditions from sub-Mediterranean to Arcto-Alpine climate contributed to a high floristic and vegetation diversity of Metohian Prokletije Mt. (Lakušić, 1968; Stevanović, 1992a; 1992b; Stevanović et al., 1995; Janković, 1998a; Amidžić & Panjković, 2003; Tomović, 2007).

Intensive research on high-mountain flora and vegetation of Prokletije, including Metohian Prokletije, has started in 1960s (Janković, 1958; Lakušić, 1968; Janković, 1972; Amidžić & Stevanović, 1997; Janković, 1998b; Amidžić & Krivošej, 1998; Amidžić & Belij, 1998; Amidžić, 2003; Pavlović-Muratspahić, 2003; etc). High-mountain zone above the upper forest border is particularly distinguished by the richness and diversity of flora and the presence of endemic plant taxa. Regarding the high floristic richness and endemism, forest ecosystems and vegetation of subalpine and alpine zone are particularly outstanding due to their refugial character in Pleistocene (Stevanović, 1996).

A special place in the high-mountain flora of Metohian Prokletije Mt. is occupied by a significant number of Balkan endemic taxa, making them one of the most important Balkan and European floristic diversity centers, along with the Šar Mt. (Amidžić et al., 2013; Tomović et al., 2014). The majority of endemic taxa are largely related to the communities of mountain pine (*Pinus mugo* Turra) which are located in a specific transition zone (so-called combat zone) between the upper forest limit and high mountain bushy and herbaceous vegetation (Janković & Amidžić, 2003).

Although a comprehensive floristic analysis of the endemic plant taxa in Metohian Prokletije has been provided by Amidžić et al. (2003; 2013), so far, no single research has been conducted and published concerning Balkan endemics within this transitional dwarf mountain pine scrub vegetation. Therefore, primary aim of this study is to provide a list of 118 Balkan endemics that were recorded within given investigated area and to present their phytogeographical (classification into major chorological groups and floristic elements) and ecological characteristics as well as syntaxonomy overview of the mountain pine communities at the upper limit of the tree line.

MATERIAL AND METHODS

Study area

The Metohian Prokletije is a continuous mountain range, over 90 km long, situated in the outermost southwestern part of Serbia and bordered by the Prokletije Mountains on the northwest (Figure 1). This mountain range is composed of 24 mountain groups covering an area of over 2.000 km^2 and is divided into three relief units located in the northern, central and southern part (Knežević, 1998; Belij, 2003).

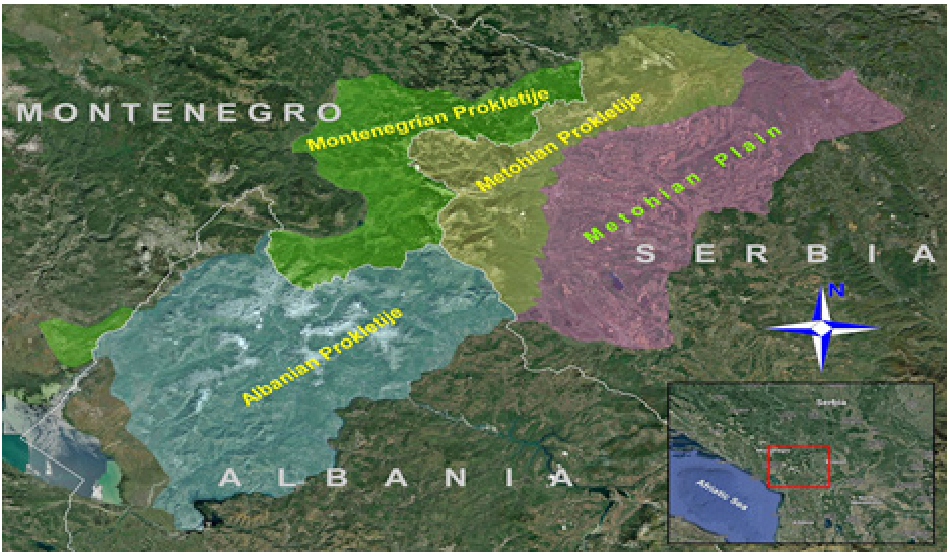

Figure 1. Geographical position of Metohian Prokletije

Owing to its specific position and the wide altitudinal range, Metohian Prokletije is characterized by a transitional climate with a per-humid sub-Mediterranean at lower elevations to a humid mountain climate of the Alpine type at higher altitudes (Stevanović & Stevanović, 1995).

In geological terms, Metohian Prokletije is composed of sediment, magmatic and metamorphic rocks (Belij, 1999; 2003), which are predominantly represented by three types of geological substrates (limestone, silicate, and serpentinite) (Šehovac, 2003). As a result of geological heterogeneity and variations of climatic conditions along the vertical profile, a complex pedology cover was formed. Soils vary from alkaline on carbonate rocks and serpentinite to acidic on siliceous rocks (Antonović, 2003).

These high mountainous areas are dominated by coniferous forests of endemorelict Macedonian pine, molika (*Pinus peuce* Grsb.) and Bosnian Pine, munika (*Pinus heldreichii* Crist). At very high altitudes over 2,000 m, above the tree line, there is a pronounced belt of transitional shrubby vegetation of mountain pine (*Pinus mugo*) (Janković and Amidžić, 2003).

Analysis of floristic data

An inventory and floristic analysis of endemic plant species found in communities of mountain pine (*Pinus mugo* Turra) within Metohian Prokletije was performed based on intensive long-standing field study conducted sporadically from 1995 to 2015. Literature data and herbarium materials are deposited in the Herbarium of the Institute for Nature Conservation of Serbia and the Institute for Nature Conservation of Vojvodina province.

The determination of endemic taxa is made using standard regional and national floristic literature such as: Josifović (1964–1977), Sarić (1986; 1992), Tutin et al. (1968–1980), Tutin et al. (1993), Jordanov/Йорданов (1963–1979), Velčev/Велчев (1982–1989), Micevski/Мицевски (1985–2005), and Jávorka and Csapody (1975).

The classification of floristic elements follows Gajić (1980), Meusel et al. (1965; 1978), Meusel and Jäger (1992) and corresponds to the phytogeographical regionalization of the Balkan peninsula into the floristic provinces given by Horvat et al. (1974), additionally modified for the territory of Serbia according to Stevanović (1992a). Taxon affiliation to appropriate life forms was determined according to the system of Raunkier (1934), which was supplemented by Ellenberg and Mueller-Dombois (1974), and was adjusted for taxa in Serbia by Stevanović (1992b).

Concerning the natural habitat types, the shrub communities of *Pinus mugo* were classified according to EUNIS habitat classification (EC, 2013).

RESULTS AND DISCUSSION

Pinus mugo Turra, known as mountain pine, is a species of conifer, native to high elevation habitats (between 1,800 m.a.s.l. to 2,600 m.a.s.l.) which range from Southwestern to Central and Eastern Europe. In Serbia, scrub mountain pine communities are found only on a few localities on high mountains of east, southeast and southern Serbia – Stara Mountain, Suva Mountain, Šar Mountain and Metohian Prokletije (Jovanović, 1955a, 1995b; Janković and Bogojević, 1974; Mišić et al., 1978; Janković, 1981; Rexhepi, 1991; Jovanović, 1992; Stevanović et al., 1994).

On Metohian Prokletije *Pinus mugo* is growing within the forest zone and above the timberline on open spaces. Below the upper forest limit as subedificatory, it grows in an adequate community (forest of *Pinus heldreichii*, *Pinus peuce*, *Picea abies*, *Abies alba*, *Sorbus aucuparia*), and it may form the shrub layer or grow individually, mainly in the limestone and dolomites. The soils on limestone bedrock are chernozems whereas on silicate rocks there are rankers and shallow acid-brown soils (Janković, 1998b).

The flattened physiognomy of the dwarf mugo pine tree growing up to 5 m and with laying, intertwined branches (Ballian et al., 2016), has emerged as a result of adaptation to the strong winds and deep and long-lasting snow cover, climatic conditions occurring at the above-mentioned elevations.

The dwarf pine is also known as a pioneer species and a hasmophyte fully adapted to rocky habitats and different types of geological substrates, covering steep, exposed and often barren slopes (Ellenberg 1988; Vidaković, 1991). Owing to its extensive root system, *Pinus mugo* has an ability to consolidate loose soils which makes it superior in reducing soil erosion in comparison with other pines (Ballian et al., 2016).

Syntaxonomic analysis

In terms of syntaxonomy, community of mountain pine belongs to the class *Vaccinio-Piceetea* Br.-Bl. 1939, order *Vaccinio-Piceetalia* Br.-Bl. 1939, and alliance *Pinion mugi* Pawl. 1928.

On the basis of previous floristic research, communities of mountain pine, which were described within this mountain complex, include:

Wulfenio carinthiacae-Pinetum mugi **Grebenščikov 1943. (syn: *Wulfenio-Pinetum mugi* (Grebenščikov 43) Janković & Bogojević 1967)** endemo-relict community on the Koprivnik, Streočka Mountain, Starac and Rusolija, mainly at an altitude of 2,040 to 2,100 m. The community occurs on calcareous and siliceous bedrocks. The soils can be designated as humus-silicate with the transition to the organic-mineral rendzinas on the limestone deposits. The stands occupy steep north-western, northern and western slopes with an inclination of over 30° (Janković and Bogojević, 1967).

Pinetum mugi **Janković 1972. (syn: *Pinetum mugi typicum* Janković 1972)** community on the Mokra gora, Hajla, Rusolija, Koprivnik, Lumbardska Mountain, Bogdaš, Nedžinat, Maja Rops, Štedim, Streočke Mountain, mainly at an altitude of 1,700 to 2,300 m. These monodominant communities prevail on the limestone terrains, mostly on skeletal mountain soils or well developed rendzinas (Amidžić et al., 2003).

Homogyno alpinae-Pinetum mugi **Pavlović-Muratspahić & Janković 1998. (syn: *Homogyno-Pinetum mugi-Vaccinietum* Pavlović-Muratspahić & Janković 1998)** community on the Mokra gora, Hajla, Rusolija, Koprivnik, Lumbardska Mountain, Štedim and Maja Rops, mainly at an altitude of 1,700 m to 2,150 m. The community is developed on siliceous bedrock, occurring on the slopes with distinct inclinations and aspects (Šibík et al., 2005).

Pinetum mughi-Geum bulgaricum **Amidžić, 1997. (syn: *Geo bulgarici-Pinetum mugi* Amidžić 2003)** community on the Nedžinat, Lumbardska Mountain, Bogdaš, Koprivnik, Rusolija and Hajla, mainly at an altitude of 2,020 m to 2,340 m. The community is well developed on limestone–dolomite rocks and shallow brown soils. The stands mainly occupy cold northeastern slopes, with an inclination of 5º to 75º (Amidžić, 1997).

Taxonomic analysis

Plant communities dominated by *Pinus mugo* are characterized by a great richness of plant species and a high percentage of endemism. In the area of Metohian Prokletije, 283 endemic species and subspecies have been registered so far (Amidžić et al., 2013), out of which 118 (42%) can be found within these communities of mountain pine. Among these, one belongs to Gymnospermae taxa while the remaining 117 are Angiospermae (Dicotyledones class with 107 plant taxa and Monocotyledones class with ten plant taxa).

In a taxonomic sense, endemic taxa of this transitional zone between the forest and herbaceous highland vegetation belong to 31 families. The highest number of endemic taxa within the zone of mountain pine belongs to the families Caryophyllaceae (16 sp.), Asteraceae (11 sp.), Fabaceae (10 sp.), Scrophulariaceae (9 sp.), Saxifragaceae (6 sp.), Brassicaceae (6 sp.), Lamiaceae (6 sp.), Poaceae (6 sp.), Violaceae (5 sp.) Campanulaceae (5 sp.) and Rosaceae (5 sp.). The most species-rich genera are *Saxifraga* (6 sp.), *Dianthus*, *Viola* (5 sp.), *Pedicularis* (4 sp.), *Heliosperma*, *Silene*, *Trifolium*, *Hieracium*, *Sesleria* and *Festuca* (each with 3 sp.).

Phytogeographical analysis

The chorological spectrum shows that the Balkan endemic taxa of this zone predominantly belong to the South-European mountainous (SEM), Central-European mountainous (CEM) chorological groups, and to a lesser degree to the Mediterranean-Submediterranean (MED-SMED) chorological group.

The prevalence of endemic orophytes from the SEM and CEM chorological groups, demonstrates a decisive influence of mountainous areas of these floristic chorions on the florogenesis and plants preservation during the Pleistocene periods of glaciations (Stevanović, 1996; Tomović et al., 2014).

Phytogeographical analysis of the Balkan endemic taxa in the investigated area indicates that the most numerous floristic elements of the CEM and SEM groups are: 1) Dinaric-Scardo-Pindhian-Moesian, 2) Dinaric-Scardo-Pindhian, 3) Dinaric, and 4) Dinaric-Moesian elements. Dinaric-Scardo-Pindhian-Moesian elements are the most abundant with 44 taxa (37%), followed by Dinaric-Scardo-Pindhian with 40 taxa (34%), Dinaric with 24 taxa (20%) and Dinaric-Moesian elements represented with only ten taxa or 9% of the overall chorological spectrum of the Balkan endemic flora in the study area (Figure 2).

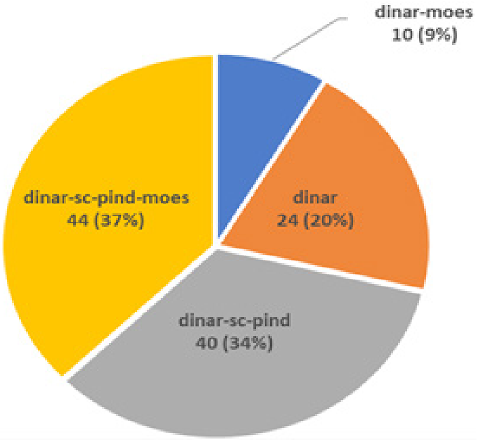

Figure 2. Chorological spectrum of the Balkan endemic flora within communities of mountain pine (*Pinus mugo* Turra) at Metohian Prokletije
Legend: dinar-sc-pind-moes – Dinaric-Scardo-Pindhian-Moesian, dinar-sc-pind – Dinaric-Scardo-Pindhian, dinar – Dinaric, dinar-moes – Dinaric-Moesian

Considering the distribution of the endemic orophytic flora belonging to SEM and CEM chorological groups in the Balkans, it can be concluded that they are widely distributed in the mountains of the Dinaric and Scardo-Pindhic systems.

The floristic list of the Balkan endemics recorded from the study area is presented in Table 1.

Table 1. The list of the registered endemic flora arranged in the alphabetical order

Floristic elements
Dinaric-Scardo-Pindhian-Moesian
1. Achillea ageratifolia (Sm.) Benth. & Hooker subsp. *serbica* (Nyman) Heimerl
2. Acinos alpinus L. subsp. *dinaricus* Šilić
3. Aconitum burnatii Gáyer subsp. *pentheri* (Hayek) Jalas
4. Alchemilla bulgarica Rothm.
5. Androsace hedraeantha Griseb.
6. Anthyllis aurea Welden
7. Armeria rumelica Boiss.
8. Astragalus glycyphylloides DC. subsp. *serbicus* (Reinchenb.) Vasić & Niketić
9. Atocion asterias (Griseb.) Tzvelev
10. Campanula persicifolia L. subsp. *sessiliflora* (C. Koch) Velen.
11. Carduus scardicus (Griseb) Wettst.
12. Cerastium malyi (Georgiev) Niketić subsp. *Malyi*
13. Cicerbita pancicii (Vis.) Beauverd
14. Corydalis solida (L.) Clairv. subsp. *incisa* Lidén
15. Crocus veluchensis Herbert
16. Dactylorhiza cordigera (Fries) Soó subsp. *bosniaca* (G. Beck) Soó
17. Dianthus cruentus Griseb. subsp. *Cruentus*
18. Dianthus tristis Velen.
19. Festuca koritnicensis Hayek & Vetter
20. Heliosperma pusillum (Waldst. & Kit.) Hoffmanns. subsp. *albanicum* (K. Malý) Neumayer
21. Knautia midzorensis Form.
22. Lamium garganicum L. subsp. *pictum* (Boiss. & Heldr.) P. W. Ball
23. Linum capitatum Kit. ex Schult. subsp. *capitatum*
24. Melampyrum scardicum Wettst.
25. Minuartia bosniaca (G. Beck) K. Malý
26. Myosotis alpestris F. W. Schmidt sups. *suaveolens* (Waldst. & Kit.) Strid
27. Onobrychis montana DC. subsp. *scardica* (Griseb.) P. W. Ball.
28. Pedicularis brachyodonta Schlosser & Vuk. subsp. *grisebachii* (Wettst.) Hayek
29. Phyteuma pseudorbiculare Pant.
30. Pinguicula balcanica Casper
31. Pinus peuce Griseb.
32. Saxifraga rotundifolia L. subsp. *chrysosplenifolia* (Boiss.) D. A. Webb.
33. Sesleria comosa Velen.
34. Sesleria latifolia (Adamović) Degen
35. Sedum alpestre Vill. subsp. *erythraeum* (Griseb.) ‘t Hart
36. Stachys scardica (Griseb.) Hayek
37. Thymus praecox Opiz subsp. *jankae* (Čelak.) Jalas
38. Thymus praecox Opiz subsp. *zygiformis* (H.Braun) Jalas
39. Trifolium medium subsp. *balcanicum*
40. Trifolium velenovskyi Vandas.

41. *Viola aetolica* Boiss. & Heldr. subsp. *denticulata* Tomović & Niketić
42. *Viola grisebachiana* Vis.
43. *Viola macedonica* Boiss & Heldr. subsp. *macedonica*
44. *Viola orphanidis* Boiss.

Dinaric-Scardo-Pindhian

1. *Achillea abrotanoides* (Vis.) Vis.
2. *Alchemilla amphiargyera* Buser
3. *Alyssum markgrafii* O. E. Schulz ex Markgraf
4. *Alyssum scardicum* Wettst
5. *Amphoricarpos autariatus* Blečić & E. Mayer subsp. *bertisceus* Blečić & E. Mayer
6. *Asperula doerfleri* Wettst
7. *Athamanta turbith* (L.) Brot. subsp. *haynaldii* (Borbás & Uechtr.) Tutin
8. *Cardamine carnosa* Waldst. & Kit.
9. *Cerastium decalvans* Schlosser & Vuk. subsp. *decalvans*
10. *Draba korabensis* Kümmerle & Degen ex Jáv.
11. *Draba kuemmerlei* Stevanović & D. Lakušić
12. *Erysimum linariifolium* Tausch
13. *Festuca halleri* All. subsp. *scardica* (Griseb.) Markgr.-Dannenb.
14. *Galium breviramosum* F. Krendl
15. *Gentianella albanica (Jáv.) J. Holub*
16. *Hieracium gymnocephalum* Pant. subsp. *gymnocephalum*
17. *Lilium carniolicum* Bernh ex Koch subsp. *albanicum* (Griseb.) Hayek
18. *Lonicera formanekiana* Halácsy subsp. *formanekiana*
19. *Lotus stenodon* (Boiss. & Heldr.) Heldr.
20. *Minuartia graminifolia* (Ard.) Jáv. *clandestine* (Portenschl.) Mattf.
21. *Oxytropis dinarica* (Murb.) Wettst. subsp. *dinarica*,
22. *Oxytropis halleri* Bunge ex Koch subsp. *кorabensis* (Kümmerle & Jáv.) Chrtek
23. *Pimpinella serbica* (Vis.) Drude
24. *Plantago reniformis* G. Beck
25. *Polygala croatica* Chodat
26. *Potentilla montenegrina* Pant.
27. *Potentilla speciosa* Willd. subsp. *illyrica* Soják
28. *Saxifraga blavii* (Engler) G. Beck
29. *Saxifraga federici-augusti* Biasol. subsp. *federici-augusti*
30. *Saxifraga scardica* Griseb.
31. *Saxifraga taygetea* Boiss. & Heldr.
32. *Scrophularia bosniaca* G. Beck
33. *Sempervivum kosaninii* Praeger
34. *Sempervivum macedonicum* Praeger
35. *Sesleria wettsteinii* Dörfler & Hayek
36. *Silene parnassica* Boiss. & Spruner subsp. *serbica* (Adamović & Vierh.) Greuter
37. *Silene schmuckeri* Wettst.
38. *Trifolium wettsteinii* Dörfler & Hayek
39. *Valeriana bertiscea* Pančić
40. *Viola elegantula* Schott

Dinaric

1. *Aquilegia blecicii* Podobnik
2. *Astragalus fialae* Degen
3. *Crepis baldaccii* Halácsy subsp. *albanica* Jáv.
4. *Dianthus integer* Vis. subsp. *integer*
5. *Edraianthus montenegrinus* Horák
6. *Euphorbia montenegrina* (Bald.) K. Malý ex Rohlena
7. *Euphorbia pancicii* G. Beck.

8. *Fritillaria messanensis* Rafin. subsp. *gracilis* (Ebel) Rix
9. *Heliosperma macranthum* Pančić
10. *Heliosperma pusillum* (Waldst. & Kit.) Hoffmanns. subsp. *monachorum* (Vis. & Pančić) Niketić & Stevanović
11. *Hieracium bertisceum* Niketić
12. *Hieracium pichleri* A. Kerner subsp. *chromoneurum* Zahn
13. *Knautia dinarica* (Murb.) Borbás subsp. *dinarica*
14. *Ligusticum albanicum* Jáv.
15. *Pedicularis brachyodonta* Schlosser & Vuk. subsp. *montenegrina* D.A. Webb
16. *Pedicularis ernesti-mayeri* Stevanović, Niketić & D. Lakušić
17. *Ranunculus concinnatus* Schott
18. *Rhinanthus asperulus* (Murb.) Soó
19. *Rhinanthus rumelicus* Velen.
20. *Saxifraga prenja* G. Beck
21. *Thlaspi dacicum* Heuffel subsp. *montenegrinum* (F. K. Meyer) Greuter & Burdet
22. *Valeriana pancicii* Halácsy & Bald
23. *Verbascum nicolai* Rohlena
24. *Wulfenia blecicii* Lakušič subsp. *blecicii*

Dinaric-Moesian

1. *Asperula aristata* L. subsp. *condensata* (Heldr. Ex Boiss.) Ehrend. & Krendl
2. *Campanula patula* L. subsp. *epigaea* (Janka) Hayek
3. *Dianthus microlepis* Boiss. subsp. *microlepis*
4. *Edraianthus jugoslavicus* Lakušić
5. *Festuca panciciana* (Hackel) K. Richter
6. *Geum bulgaricum* Pančić
7. *Pedicularis heterodonta* Pančić
8. *Senecio wagneri* Degen
9. *Silene sendtneri* Boiss. subsp. *sendtneri*
10. *Stachys alpina* L. subsp. *dinarica* Murb.

Life form spectrum

The life-form spectrum of the endemic flora within communities of mountain pine in the study area is presented in Figure 3. Analysis of present life forms of endemic taxa reveals a high percentage of hemicryptophytes (64 taxa, i.e., 54.24%) and chamaephytes (45 taxa, i.e., 38.14%), with a significantly lower percentage of geophytes (5 taxa, 4.24% respectively), therophytes (2 or less than 2%) and phanerophytes (2 i.e., less than 2%).

Given the fact that life forms are adapted to the unfavorable period of the year in different ways, the biological spectrum provides a good insight into the ecological conditions (in particular climatic and edaphic) in which a certain phytocenosis has been developed (Stevanović and Janković, 2001). This ratio of life forms with the high percentage of hemicryptophytes and chamaephytes present in the study area indicates extreme environmental conditions, specifically reflected at low temperatures, mostly occuring during the year in high mountain regions. According to Raunkier (1934) hemicryptophytes as herbaceous perennials and chamaephytes as low-growing perennials and dwarf shrubs have their overwintering buds at the soil surface or close to the ground, where they are protected by the snow cover.

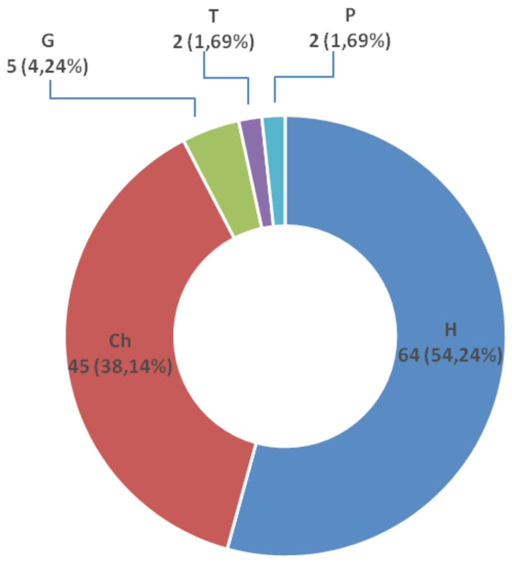

Figure 3. Life-form spectrum of the endemic flora recorded in studied area of Metohian Prokletije
Legend: H – Hemicryptophytes, Ch – Chamaephytes, G – Geophytes, T – Therophytes, P – Phanerophytes

Conservation status and importance

According to EUNIS habitat classification (EC, 2013) shrub communities of dwarf pine are categorized as Subalpine *Pinus mugo* scrub (EUNIS code F2.4). In addition, scrub formed by dwarf mountain pine are listed in Anex I of EU Habitats Directive (Directive 92/43/EEC) with Natura 2000 code 4070* – Bushes with *Pinus mugo* and *Rhododendron hirsutum* (*Mugo-Rhododendretum hirsuti*) and are assessed as natural habitat types of community interest where conservation requires the designation of special areas of conservation.

At the national level, *Pinus mugo* scrub (B 6.11) are considered as rare, endangered and fragile habitat types of high priority for protection (Anonymous, 2010b) included within protected area – NP Prokletije, whereas *Pinus mugo* Turra subsp. *mu*go is declared as strictly protected species (Anonymous, 2010a).

During the last decades, plant communities with *Pinus mugo* and associated endemic species were for the most part threatened by illegal logging of forests, human-caused fires, overexploitation, uncontrolled and unplanned constructions which have resulted in the habitat degradation and fragmentation or loss of the forest ecosystems (Menczer et al., 2018). The most critical situations are in steep terrain where deforestation intensified soil erosion. Some of the endemic species within dwarf pine communities are known as medicinal, aromatic and industrial plants wherefore they are subjected to uncontrolled collection and thereby in danger of becoming extinct.

Considering conservation importance, it is worth mentioning that *Pinus mugo* and its shrubby high-mountain communities in transition zone above the timberline form a characteristic vegetation belt that used to play a protective role in preventing avalanches, landslides and soil erosion (Janković, 1958; Alexandrov et al., 2011; Alexandrov et al., 2019).

CONCLUSIONS

Although Metohian Prokletije has been a subject of several intensive floristic and vegetation researches over the last several decades, there is still a lot to be investigated, particularly regarding the floristic diversity and endemism of its high mountainous areas. Apart from remarkable floristic richness, the special value of this mountain system is Balkan endemic vascular flora created as a result of diverse climatic, edaphic and orographic conditions. The high number of endemic flora (118) was recorded as well within communities of mountain pine (*Pinus mugo* Turra) in the so-called "battle zone" between coniferous forests on one side and low growing shrubs and herbaceous highland vegetation, on the other, above the tree line elevation.

Significant presence of Dinaric-Scardo-Pindhian-Moesian, Dinaric-Scardo-Pindhian and Dinaric floristic elements within the prevailing CEM and SEM chorological groups demonstrates the close phytogeographical connections of corresponding provinces and their strong impact on the genesis of the orophytic Balkan endemic flora of the investigated area.

The influence of the cold, windy and snowy mountainous climate is expressed in the prevalence of hemicryptophytes and chamaephytes in the biological spectrum.

The results of this study contribute to the overall knowledge of the endemic plant richness of the Metohian Prokletije and confirm their status as one of the major European centers of the Balkan floristic endemism.

The existence of a large number of endemic plants, along with both ecological and protective role of the dwarf pine communities on steep, mountainous terrain, in soil erosion control, water retention and maintenance of the forest biodiversity, including their economic benefits as well, are sufficient criteria to ascribe high conservation importance to this transition zone of *Pinus mugo* communities over the tree limit.

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ОРИГИНАЛНИ ЧЛАНАК

ЕНДЕМСКА ФЛОРА ЗАЈЕДНИЦЕ БОРА КРИВУЉА (*Pinus mugo* Turra) НА МЕТОХИЈСКИМ ПРОКЛЕТИЈАМА

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РЕЗИМЕ: Због свог специфичног положаја на ободу Средоземног мора, сложених и разноврсних геоморфолошких, геолошких, орографских и климатских одлика, Метохијске Проклетије су означене као један од главних центара балканског флористичког ендемизма у Европи. У раду су приказани резултати вишегодишњих интензивних флористичких истраживања ендемске флоре Метохијских Проклетија, с посебним освртом на њихове високопланинске области изнад горње шумске границе, где су концентрисане заједнице бора кривуља (*Pinus mugo*). Ови екосистеми представљају прелазну зону између четинарских шума и високопланинске жбунасте и зељасте вегетације, на надморској висини од 1.700 до 2.400 m. На основу досадашњих детаљних теренских истраживања, у оквиру ових заједница бора кривуља забележено је 118 балканских ендемичних биљних таксона. Фитогеографска анализа показује да ендемични таксони претежно припадају Јужноевропској планинској (SEM) и Централноевропској планинској (CEM) хоролошкој групи са следећим учешћем флористичких елемената: 1) Динарско-скардо-пиндско-мезијски (44 таксона или 37%), 2) Динарско-скардо-пиндски (40 таксона или 34%), 3) Динарски (24 таксона или 20%) и 4) Динарско-мезијски (10 таксона или 9%). Биолошки спектар карактерише доминантна заступљеност хемикриптофита и хамефита. Добијени подаци о присуству бројних ендемичних биљака на проучаваном подручју могу послужити као одлична основа за даља флористичка истраживања, а информације о еколошком значају као и конзервационом статусу угрожених биљних заједница бора кривуља имају за циљ подстицање ефикасније заштите биодиверзитета и одрживог управљања природним ресурсима.

КЉУЧНЕ РЕЧИ: ендемска флора, заједнице бора кривуља (*Pinus mugo*), Метохијске Проклетије

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ORIGINAL ARTICLE

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CHEMICAL COMPOSITION OF *Moringa oleifera* AND CRUCIFEROUS PLANTS AND THEIR ANTI-INFLAMMATORY PROPERTIES

ABSTRACT: *Moringa oleifera* Lam. is a tropical tree that has been traditionally used for food, medicine and industrial purposes. All parts of the tree have been utilized and have biological activities, including anti-inflammatory, antibacterial, antioxidant, antidiabetic, wound healing, cardiovascular, anti-asthmatic, hepatoprotective, protection of the central nervous system and more. The leaves and seeds of moringa are particularly rich in phytochemicals, including glucosinolates and isothiocyanates (ITCs). Glucosinolates are inactive precursors that are hydrolyzed into bioactive ITCs. Isothiocyanates, such as 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate (moringa isothiocyanate 1, or MIC-1), are known for their diverse biological activities, including anti-inflammatory, antioxidant and anticancer properties. The unique sugar moiety in moringa's ITCs contributes to its stability and distinct properties compared to other cruciferous vegetables. *In vitro* models, such as the TIM-1 model and *in vivo* murine models demonstrate MIC-1's bioaccessibility and bioavailability, with no statistical difference in state. Inflammation, as a defense mechanism, is implicated in a wide range of diseases such as diabetes, cancer, obesity, cardiovascular disease, arthritis and multiple sclerosis. *In vivo* models were used to evaluate the efficacy of MIC-1 against acute skin inflammation, which inhibited the inflammatory pathway. Additional studies into *Moringa oleifera* seed extract effects on acute inflammation can contribute to future development of therapies managing chronic inflammatory conditions.

KEYWORDS: *Moringa oleifera*, cruciferous plants, inflammation, isothiocyanate, glucosinolate

INTRODUCTION

The use of naturally derived plant products for medicine, while seemingly gaining in popularity nowadays, has been around for thousands of years.

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Since ancient times, people have been turning to nature for the treatment of ailments using bark, seeds, fruit bodies and many other plant parts (Petrovska, 2012). The first documented use of plants for medicine goes as far back as 2600 BC. Written on clay tablets in Mesopotamia, there were descriptions of the use of materials including oils of *Cedrus species* (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora species* (myrrh) and *Papaver somniferum* (poppy) (Gurib-Fakim, 2006). All of these plant oils are still used today in some form of treatment for a variety of conditions. It is of interest that many natural plant compounds, those that are safe for human consumption, can be used in place of, or in addition to modern pharmaceuticals since most have little to no harmful side effects and are cheaper to source and safer to consume over longer periods of time (Gossell-Williams et al., 2006). The general terms for commercial plant extracts used to support the normal functioning of the human body are "dietary supplements" or "botanicals" (Sachdeva et al., 2020). As the familiarity with these botanicals increases, researchers are studying their effects in treating a variety of diseases, thereby narrowing the gap between natural / traditional medicines and modern pharmaceuticals.

Moringa oleifera Lam.

In tropical and sub–tropical environments, among the abundant plant life, there is a drought resistant tree that can grow to about 7–10 meters with sparse foliage and distinctive stick-like pods, or drumsticks, as they are frequently referred to (Makkar and Becker, 1997). This *Moringa oleifera* tree, commonly referred to as moringa, is a member of the Moringaceae family, consisting of 13 known species. All of them, including *Moringa oleifera*, are traditionally used for food, medicine or industrial purposes, including their the pods, seeds leaves, bark and roots (Senthilkumar et al., 2018). Moringa leaves are traditionally used for nutrition. Dried leaves have 30.3% protein, 3.7% calcium, 0.3% phosphorus, 1.5% potassium, 0.2% sodium, 0.6% sulfur, 8.2% copper, 13.0 mg/kg zinc, 86.8 mg/kg manganese, 490 mg/kg iron, and 363 mg/kg selenium (Moyo et al., 2013). They are also rich in antioxidants such as beta carotene, vitamin C, quercetin and chlorogenic acid which is used to lower blood sugar (Paikra et al., 2017). Even before reports of their phytochemical analysis, the leaves have been used as a poultice or paste and applied externally to sores and sprains, and consumed for the treatment of anemia, menstrual irregularities, stomach aches, constipation and piles (Pandey, 2012). The leaves have also been used to combat malnutrition in infants and nursing mothers and serve as a short-term alternative to chemoprophylaxis (disease prevention) in livestock (Moyo et al., 2013). Studies have identified about 16 bioactives in a methanolic extract of the leaves. The present phytochemicals were found to be phenols, flavonoids, saponins, anthocyanins and alkaloids. With the exception of the phenols and alkaloids, all these phytochemicals were found in higher concentrations in the seeds of moringa as compared to the leaves (Dhakad et al., 2019).

Long established uses of moringa seeds include filtering the sediment for water purification and arsenic removal (Paikra et al., 2017, Muhl et al., 2016); adding defatted seed meal to sheep diet in order to improve rumen fermentation (Ben Salem and Makkar, 2009); supplementing the poor diets of cows with moringa protein to increase milk production (Reyes Sánchez et al., 2006); and extracting the oil for cooking and frying due to its optimal stability (Abdulkarim et al., 2007).

Similar to the leaves, the phytochemical composition of the seeds includes both essential and nonessential amino acids including alanine, glutamate, glycine, lysine, threonine and methionine. The seeds are also rich in calcium, phosphorus, iron, potassium, magnesium, zinc, copper and manganese (Saa et al., 2019). Due to the plethora of phytochemicals, the biological activities reported in both the leaves and seeds of the plant include anti-inflammatory, antibacterial, antioxidant, radical scavenging, antidiabetic, wound healing, antiepileptic, anticonvulsant, cardiovascular, anti-hypertensive, anti-fertility, anti-urolithiatic, anti-asthmatic, hepatoprotective and protection of the central nervous system (Paikra et al., 2017; Arulselvan et al., 2017).

While the leaves and seeds are the most utilized parts of the plant, it is worth noting that all parts of the plant contain some bioactives and have been used for both medicinal and traditional purposes. An extract of the roots of moringa was reported to contain an active and powerful antibiotic and fungicidal, pterygospermin (Kou et al., 2018). An alcoholic extract of the bark contains a lesser amount of the main glucosinolates then found in the seeds and leaves, 4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate; a hydro-alcoholic extract of the flowers contains antioxidants such as quercetin, kaempferol, isoquercetin, ascorbic acid; an aqueous and hydroalcoholic extract of the stem has 4-hydroxyl mullein, vanillin, octacosonoic acid, beta- sitosterone and beta-sitosterol, all used for cosmetic or medicinal purposes (Paikra et al., 2017). For all its multipurpose uses and vast number of nutrients found in all parts of the plant, moringa is unsurprisingly often called ‘the Miracle Tree’.

Moringa oleifera’s Glucosinolates and Isothiocyanates

Similar to the Moringaceae family, the phytochemicals in the members of the Brassicaceae family range from folic acid, phenolics, carotenoids, selenium, ascorbic acids, and a group of compounds called glucosinolates (Sharma et al., 2016). Glucosinolates are β-thioglucoside N-hydroxysulfates, (Z)-(or cis)-N-hydroximinosulfate esters, or S-glucopyranosyl thiohydroximates, with a side chain (R) and a sulfur-linked β-D-glucopyranose moiety (Fahey et al., 2001).

Glucosinolates are sulfur-containing secondary metabolites that are precursors to the bioactive isothiocyanates (ITCs), a product of the hydrolysis reaction that is mediated by myrosinase (β-thioglucosideglucohydrolase, EC 3.2.3.1). The conversion also occurs by the microflora of the intestines (De Nicola et al., 2013). The 120 known glucosinolates are biologically inactive molecules that could have adverse effects on animals that ingest them in high amounts (Tripathi and Mishra, 2007). They make up roughly 1% of dry weight in the tissue of most *Brassica* vegetables (Fahey et al., 2001). The glucosinolates co-occur with the enzymes that catalyze their conversion into their isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidine counterparts. The products are dependent on conditions including pH, metal ions and other protein elements, however, isothiocyanates are the most commonly produced metabolites of glucosinolates (Bones and Rossiter, 2006).

Isothiocyanates are a class of secondary metabolites that have a characteristically bitter taste and pungent odor, mostly found in the Brassicaceae family of plants (Brown and Hampton, 2011). They are thioglycoside conjugates of glucosinolates and are formed when the cells of the plant are damaged, (for example by chewing) and an enzyme called myrosinase is released. This enzyme aids in catalyzing the hydrolysis of glucosinolates via the Lossen rearrangement-cleaving the thio-linked glucose and leaving the aglycone (Hecht, 1995; Waterman et al., 2014). Research into the biological effects of isothiocyanates has ranged from metabolism regulation, carcinogenesis, cardiovascular protection, protection of the central nervous system, diabetic nephropathy and neuropathy, antimicrobial and restoration of skin integrity (Dinkova-Kostova and Kostov, 2012; Padla et al., 2012). More specifically, the studies have shown that a number of naturally derived ITCs, including the ITCs from broccoli, watercress, and synthetic analogs have been effective at inhibiting chemically induced tumors in organ sites of rodents including the pancreas, stomach, bladder, colon and esophagus (Wu et al., 2009). Moreover, approximately 20 natural and synthetic isothiocyanates were shown to inhibit chemically induced carcinogenesis *in vivo* and *in vitro* (Wu et al., 2009). One study on anti-cancer activities of botanicals demonstrated that *Moringa oleifera* extract had a higher rate of tumor cell death than groups treated by sulforaphane alone (Ghaed Amini et al., 2023). The consumption and bioavailability of sulforaphane, an isothiocyanate, has been thoroughly investigated and is well documented and serves as a gold standard for ITC research (Oliviero et al., 2014; Oliviero et al., 2018; Angelino and Jeffery, 2014; Abbaoui et al., 2018; Thornalley, 2002).

Many isothiocyanates are volatile compounds due to the isothiocyanate (-N=C=S) group. They are known for their biological functions such as being a herbivore deterrent, fungicidal, bactericidal, nematocidal and being allelopathic and in the treatment of many ailments (Fahey et al., 2018). In moringa, there are 4 isothiocyanate products, with MIC-1, 4-(α-L- rhamnopyranosyloxy) benzyl isothiocyanate, being the most abundant. The other three, MIC-2, MIC-3, MIC-4 are named based on the position of an acetyl group on the R2, R3 or R4 position. These MICs are produced in lower amounts, but all four contain an additional sugar moiety in the aglycone/ITC part of the molecule, which differs from the ITCs of other cruciferous vegetables (Waterman et al., 2014). Figure 1 depicts the aromatic ring and rhamnose moiety on MIC-1 that sets it apart from other isothiocyanates as a high-yielding, stable, white powder, as opposed to a low-yielding, volatile oil (Thornalley, 2002; Rojas-Silva et al., 2015; Borgonovo et al., 2020), making MIC-1 a superior ITC as compared to other cruciferous vegetables.

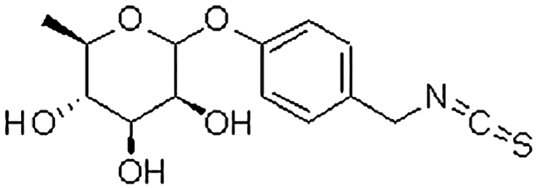

Figure 1. Structure of MIC-1 (4-Hydroxybenzyl isothiocyanate rhamnoside or 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate). The distinctive sugar moiety attached is proposed to allow MIC-1 to be a stable, high-yielding white powder.

The consumption of fruits and vegetables with high nutritional value like moringa and other cruciferous vegetables like cabbage, watercress and brussels sprouts are associated with a reduced risk of diseases (Vermeulen et al., 2008). The most well studied isothiocyanate, sulforaphane, is an isothiocyanate found in high amounts in broccoli, well known cruciferous plant. Its precursor glucoraphanin is converted via myrosinase into sulforaphane. Studies have shown that eating raw broccoli leads to higher amount of sulforaphane and its conjugates in the blood and urine of persons rather than when they consume cooked broccoli (Vermeulen et al., 2008).

Bioavailability and Bioaccessibility of ITCs

Understanding the bioavailability of any pharmacologically active compound is an important step for its clinical development. Bioaccessibility is the first stage in defining the bioavailability of a compound and is performed *in vitro,* while bioavailability testing is done *in vivo*, as it requires the blood system (Rein et al., 2013). It is important to understand how MIC-1, an isolated pure compound, and moringa seed extract (MSE) perform within the human digestive system by defining the bioaccessibility and later, its bioavailability. The investigation into whether isothiocyanates are both bioavailable and bioaccessible is important for their functional validation as future dietary supplements or as conventional pharmaceuticals (Boyunegmez et al., 2015).

Bioaccessibility can be defined as “the amount of the compound that is released from the food matrix in the gastrointestinal (GI) tract that is available for absorption” (Carbonell-Capella et al., 2014).

While the human digestive system is a very complex and dynamic environment, there exist *in vitro* models that at least partially mimic the stomach and small and large intestines. Models include those that mirror stomach-to-colon (SHIME) (Molly et al., 1993) or just the colon (HMI or Gut-on-a-chip) (Marzorati et al., 2014; Kim et al., 2012) among others. While *in vivo* studies mainly sample the end point of the digestive system (urine or feces) to see the effect of a compound, intestinal *in vitro* models can rely on sampling different regions of the gut, making it possible to monitor changes at various locations (Williams et al., 2015). The multi-compartment computer-controlled TNO gastrointestinal model (TIM-1 model) simulates the *in vivo* conditions and kinetic events of the stomach, duodenum, jejunum, and ileum of the small intestines (Ribnicky et al., 2014). The dynamic digestion in TIM-1 suggests that it can stimulate continuous changes of the physiochemical conditions of the digestive system, from mouth to stomach, and it also allows for changing pH and enzyme secretion concentrations (Alminger et al., 2014). This system contains compartments representing the stomach, duodenal, jejunal and ileal parts of the gastrointestinal tract. Each of these four compartments has two glass jackets that are lined with flexible walls. It stimulates gastric emptying rate, peristaltic movements and gradual pH changes in the different compartments (Alminger et al., 2014). This model was ideal to explore the workings of MIC-1 while being "consumed" in both fed and fasted state. It was found that MIC-1 was bioaccessible, with no statistical difference in the either state. MIC-1 was also found to be bioavailable using an *in vivo* mouse model that investigated the amount of MIC-1 in blood serum over time (Wolff et al., 2020). Briefly, a subset of animals was administered MIC-1 via intravenous (IV), another subset received MIC-1 via oral gavage and a third subset received MSE via oral gavage. The IV-administered MIC-1 showed that about 20% is bioavailable and even when orally administered, unlike other isothiocyanates that are conjugated with glutathione and metabolized into mercapturic acid or bithiocarbamate products, MIC-1 remained unchanged (Janobi et al., 2006).

Bioavailability is the fraction of the consumed compound that gets into the circulatory system (blood) that can then be distributed to tissues and organs (Dima et al., 2020). It incorporates the following four components: 1) availability for absorption or bioaccessibility; 2) absorption; 3) tissue distribution; and 4) bioactivity (Harmsen, 2007). Determining the bioavailability of a botanical such as moringa using *in vivo* model is important because in order to be consumed as part of the diet, a significant therapeutic level must be sustained when ingested orally (Ting et al., 2014). Evaluating the bioavailability of MIC-1, both isolated and in MSE, was performed by orally gavaging a group of mice and collecting the blood serum at different time points. The chemical structure and properties of the compound are important to consider as they are affected by the gut physiological and physiochemical factors as the compound moves from the mouth, stomach, small intestine then large intestine and colon, under various temperature, pH conditions and enzyme secretions resulting in breaking down of the compound along the way (Ting et al., 2014).

Studies have shown that consumed isothiocyanates are conjugated to glutathione and then further metabolized into mercapturic acids, predominantly an N-acetyl cysteine conjugate, which is then excreted into the urine. Therefore, the presence of mercapturic acid in the urine indicates that isothiocyanates were ingested (Vermeulenn et al., 2008; Sivapalan et al., 2018). In rats, sulforaphane is rapidly absorbed, achieving high bioavailability at low doses after being fed fresh broccoli, with peak intracellular concentration within 3 h of doses. Oral absolute bioavailability was over 80% at the lowest dose of 0.5 mg/kg but decreased as concentrations increased (Hanlon et al., 2008).

Inflammation

The World Health Organization (WHO) reported that in the year 2000, nearly 125 million Americans live with chronic conditions which include inflammatory diseases (Pahwa et al., 2022). Inflammation, the body's defense mechanism, is involved in diseases such as diabetes, cancer, obesity, cardiovascular disease, arthritis, allergies, multiple sclerosis, and chronic obstructive pulmonary disease (Pahwa et al., 2022; De Barcelos et al., 2019).

Inflammation can be defined as the body's recognition and defense mechanism against harmful and foreign stimuli. It involves the activation of immune and non-immune cells that protect against bacteria, viruses, toxins, and infections, and can be categorized as acute or chronic, depending on the severity and length of time of the inflammatory response (Furman et al., 2019). This process involves the release of substances such as cytokines and chemokines that are responsible for the dilation and increase in permeability of capillaries, which results in tissue that is swollen and inflamed (Furman et al., 2019). This acute response contributes to the resolution of inflammation and restoration of tissue homeostasis, but if it is uncontrolled, it may become chronic and lead to a range of chronic inflammatory conditions (Chen et al., 2018).

Many inflammatory diseases are the result of acute or chronic inflammation. Acute inflammation is typically reserved to the tissue level, for example, the response to a laceration of the finger. It is characterized by redness, swelling, heat, pain and loss of the function of the tissue, due to local immune, vascular and inflammatory cell responses to the infection or injury. The microcirculatory events include changes to the vascular permeability, leukocyte recruitment and accumulation and release of inflammatory mediators (Chen et al., 2018).

Chronic inflammatory conditions include but are not limited to cardiovascular, arthritis, cancer, fatty liver, type 2 diabetes mellitus, type 1 diabetes mellitus, inflammatory bowel disease and asthma that are not limited to age, gender or socioeconomic background (Pasparakis et al., 2014).

In general, the inflammatory response process can be summarized by the following processes: cell surface pattern receptors recognizing the stimuli, activation of inflammatory pathways, release of inflammatory markers and recruitment of inflammatory cells (Chen et al., 2018). Various transcription factors and pathways are activated during this time, such as activator protein-1 (AP-1), STAT3 and TNF pathway (Pasparakis et al., 2014). AP-1 transcription factors are found to be the most important regulators in the epidermis of the skin in regulating epidermal homeostasis and diseases such as cancer (Eckert et al., 2013). Some key cytokines present during the inflammatory response include stimulators of acute phase proteins such as IL-6, IL-1β, tumor necrosis factor-β, interferon-γ, transforming growth factor-β and IL-8 (Wigmore et al., 1997). Another important transcription factor to note is NF-κβ. This transcription family has five members which include p65 (ReIA), RelB, c-Rel, NF-κβ1 and NF- κβ2. They all share some structural features such as being bound to Iκβ proteins, the inhibitor of NF- κβ, in quiescent cells (Hoesel and Schmid, 2013). It is known that NF-κB controls the expression of cytokines IL-1β and TNF, important markers in inflammation, and in turn induce their expression. Along with their expression, there are also elevated levels of cytokines, including IL-6 and IL-15, adhesion molecules and chemokines IL-8, MCP-1 and MIP-1α, all to promote the activation and recruitment of inflammatory cells (Makarov, 2000).

The skin, the largest organ of the body, is composed of three main parts, from outermost to innermost, the epidermis, the dermis and the subcutis, respectfully (Sabat et al., 2019). It provides a life- sustaining barrier between the body and the environment, restricting water loss and preventing entry of harmful microorganisms and substances (Pasparakis et al., 2014). While sometimes the cause of skin inflammation may be due to external factors, others are the results of autoimmune diseases. The development of such skin pathologies involves a possible combination of genetic susceptibility, environmental influence such as infections, smoking, drinking, or drugs and the presence of an autoantigen (Bowcock, 2005). An example of an autoimmune disease would be rheumatoid arthritis (RA). It is common, affecting about 2–3% of the population or 125 million people according to the National Psoriasis Foundation. It is characterized by inflammation and hyperplasia of the synovial fluid, cartilage and bone destruction, and even systemic features such as cardiovascular, pulmonary psychological and skeletal disorders (McInnes and Schett, 2011). The initiators of the inflammatory process are the keratinocytes in the epidermis, which receive signals and transmit them to the immune cells. The receptors sense the presence of stimuli, microorganisms for example, and produce cytokines, chemokines, and their receptors.

Examples of *in vivo* models used to evaluate the effectiveness of MIC-1 and MSE in an acute inflammatory response are a carrageenan paw edema model where the inflammation site is the hind paws of the animals. Injection of a 1% carrageenan solution elicits an inflammatory granulomatous reaction that is characterized by a production of prostaglandin, leukotriene and polymorphonuclear leukocytes (PMNs) and macrophages (Morikawa et al.,2003). The lambda form of this family of polysaccharides has historically been used as a stimulant for an *in vivo* model of a local inflammatory response. Carrageenan injection causes local edema, infiltration of white blood cells, increased levels of local PGE2, increased interleukin-8 secretion in cell tissue culture via nuclear localization of NFκB (Myers et al., 2019). The second *in vivo* model, TPA-induced ear edema model, uses 12-O-tetradecanoylphorbol-13-acetate that elicits the inflammatory response by production of reactive oxygen species (ROS) and pro-inflammatory mediators that include cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), and is closely associated with the nuclear factor-kappaB (NF-kB) and the mitogen-activated protein kinase (MAPK) pathways (Jeong et al., 2014).

Although there are similarities between *in vivo* studies performed on mice and rats and human experiments, the skin of rodents does differ from the skin of humans. The anatomical and immunological differences between murine and human skin include the following: murine skin is covered with a thick layer of hair while humans have sparse hair coverage. Mouse skin is thinner than human's and has a superficial muscle layer that contracts allowing for healing without scars while humans use re-epithelization and granulation to heal that leaves scars behind (Di Meglio et al., 2011). Mouse skin also contains specific types of dendritic cells CD207(langerin)+CD103+ dermal DCs (DDCs) and Vγ5Vδ1 T cells also known as dendritic epidermal T cells (DETCs) while humans either lack them or they have not been identified (Di Meglio et al., 2011).

While there are differences, the use of mouse skin for topical experiments is the gold standard when long and costly clinical trials are not an option. The use of CD-1 mice to investigate the topical anti-inflammatory effects of an MSE and its isothiocyanate is relevant to the further development of a topical treatment, perhaps in combination with others, for the symptoms of inflammatory conditions.

ITCs and inflammation

The sharp taste of cruciferous vegetables, the result of an active defense system, is due to the presence of isothiocyanates. These compounds possess strong anti-inflammatory, anticarcinogenic, antioxidant, antimicrobial, neuroprotective, and cardioprotective activities (Chandra et al., 2018). ITCs are well studied in vegetables such as broccoli, watercress, brussels sprouts, cabbage, Japanese radish and cauliflower. Some ITCs include sulforaphane, phenethyl isothiocyanate (PEITC), and benzyl isothiocyanate (BITC), all of which have been shown to be highly effective at reducing the risk of cancer in carcinogen animal models, and *in vitro*, limiting the growth of various cancer cells (Wu et al., 2009). While ITCs in *Moringa oleifera* are not as well studied, the literature shows that cruciferous ITCs such as PEITC and BITC are also present in moringa, in addition to its unique MIC compounds (Bose, 2007).

Studies have demonstrated the effects of isothiocyanates in alleviating inflammation in different inflammatory models. Water-extractable isothiocyanates from moringa leaves have decreased gene expression and decreased production of inflammatory markers iNOS, IL-1β, nitric oxide and TNFα *in vitro* (Waterman et al., 2014). Topically, a UVB radiation study showed that skin erythema was reduced by 40% at the sites that received a topical treatment with sulforaphane-containing broccoli extract compared to the sites that received the vehicle alone (Dinkova-Kostova and Kostov, 2012). Again, as a topical treatment, 2% moringin cream was shown to relieve neuropathic pain due to the inhibition of inflammatory pathway and blockade of voltage-gated ion channels (Giacoppo et al., 2017). Another study showed that encapsulated liposomes of naringin with phenyl isothiocyanate and sulforaphane, two isothiocyanates, were able to decrease inflammation in both an acute carrageenan paw edema model and a chronic arthritis model in rats (Mohanty et al., 2020).

CONCLUSION

In conclusion, *Moringa oleifera* contains four isothiocyanate (ITC) products, with MIC-1, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, being the most abundant in the seed of the plant. MIC differs from other ITC compounds due to the presence of a sugar moiety; this contributes to the unique property of MIC isothiocyanates to remain stable as compared to those from other cruciferous vegetables. Research has shown that MIC-1 is bioavailable and remains unchanged even when orally administered, and also has anti-inflammatory effects as seen in rat and mouse models. The findings presented in this review suggest that formulating MSE or MIC-1 into a preventative therapeutic agent may potentially be used in combination with prescribed medicine. The use of a naturally derived plant product, such as *Moringa oleifera*, incorporated into a daily regimen could be the added therapeutic in delivering health and wellness. Further investigation of the effects of *M. oleifera* seed extract and its main isothiocyanate, MIC-1 in an acute skin inflammation model are needed.

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ОРИГИНАЛНИ ЧЛАНАК

ХЕМИЈСКИ САСТАВ *Moringa oleifera* И КРСТАШИЦА И ЊИХОВА ПРОТИВУПАЛНА СВОЈСТВА

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РЕЗИМЕ: *Moringa oleifera* Lam. је дрво тропских области које се традиционално користи као храна, лек и у индустријске сврхе. Употребљавају се сви делови биљке у којима се налазе биолошки активне супстанце а које делују антиинфламаторно, антибактеријски, антиоксидативно, антидијабетски и помажу код зарастања рана. Такође позитивно делују на кардиоваскуларни систем, испољавају антиастматично и хепатопротективно дејство, штите централни нервни систем и имају још позитивних улога. Листови и семенке моринге богати су фитоједињењима, посебно глукозинолатима и изотиоцијанатима (ITC). Глукозинолати су неактивни прекурсори који хидролизом дају биоактивне изотиоцијанате. Изотиоцијанати, као што је 4-(α-Lramnozopiranoziloksi) бензил изотиоцијанат (Moringa изотиоцијанат 1, или MIC-1), познати су по својим разноврсним биолошким својствима, посебно антиинфламаторном, антиоксидативном и антиканцерогеном. Јединствени шећерни остатак присутан у изотиоцијанатима моринге доприноси њеној стабилности и даје јој посебна својства у односу на друге биљке из породице купусњача. *In vitro* модели, попут TIM-1 модела и *in vivo* експерименти с мишевима, показују да је MIC-1 лако доступан и да се добро апсорбује у организму, при чему нема статистички значајне разлике у резултатима. Упални процес, као одбрамбени механизам, јавља се код великог броја болести као што су дијабетес, канцер, гојазност, кардиоваскуларне болести, артритис и мултипла склероза. *In vivo* експерименти су коришћени за испитивање ефикасности MIC-1 у случају акутне упале коже која инхибира инфламаторне путеве. Додатна истраживања ефеката екстракта семена *Moringa oleifera* на акутне упале допринеће будућем развоју терапија за третман хроничних инфламаторних стања.

КЉУЧНЕ РЕЧИ: *Moringa oleifera*, крсташице, упала, изотиоцијанат, глукозинолат

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ORIGINAL ARTICLE

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SOIL – TO – LEAF RELATIONSHIP IN MICRO AND MACROELEMENTS CONTENT ON THE VINEYARD MICROLOCATION

ABSTRACT: The conducted research aims to ascertain the variations in macro and microelement content within the soil-to-leaf relationship in small vineyards. The vineyard block (1.2 ha), located in Sremski Karlovci, Serbia, planted with Grašac (Riesling Italico), was divided into 20 subplots. Each subplot served as an individual location for soil and leaf sampling. Soil samples were collected at three depths, while leaf sampling occurred at two phenophase (end of flowering and at ripening), with separation into petiole and blade parts. Variability of soil physico-chemical characteristics between subplots was determinate, with the greatest variability in the 30–60 cm soil layer. The soil generally displayed low levels of organic matter and available P, K, Zn and B. Erosion processes were indicated by the spatial distribution of physico-chemical parameters. Differences in nutrient contents were noted among leaf parts and phenophases, aligning with existing literature. Comparing leaf nutrient status to optimal values from literature, N and P content was found at lower limits, confirming K and B deficiencies. Identically, fertilization recommendations can be inferred from soil and foliar analyses, primarily for N, K and B. Additionally, based on soil analysis, a slight increase in P and Zn application is advisable. Established correlations among all observed variables revealed connections between soil parameters, across all depths, and nutrients in the leaf blade at the end of flowering. It is notable to say that nutrient content in soil, particularly N, K, Mn and Zn, exhibited statistically significant positive correlations with its content in the leaf blade, respectively. Further research is necessary to lay the foundation for the development of accurate and reliable criteria for diagnosing nutrition, not only for the whole species but also among grapevine leading varieties. Given the significant variations in nutrient requirements and accumulation among these genotypes, this research will be instrumental in ensuring optimal nutrient supply while minimizing deficiencies or excesses.

KEYWORDS: vineyard soil, foliar analysis, Grašac (Riesling Italico) variety

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INTRODUCTION

In viticulture, there exists a greater potential for anthropogenic influence on soil improvement, compared to the possibility of improvement intervention on other abiotic factors, such as climate. Therefore, soil management should be approached systematically, already during the establishment of the vineyard (Ninkov et al., 2017). The transfer of nutrients from soil to plants has a significant impact on yield and, more importantly, on the quality of grapes, particularly in high-quality varieties that are used for winemaking. Both nutrient deficiencies and sufficiency have a crucial influence on the quality and yield of grapes. For example, it has been found that a low level of potassium can reduce sugar content and the amount of colour-giving compounds in grapes, while a high K level can decrease must acidity. Elevated nitrogen levels can delay ripening, resulting in lower levels of anthocyanins, increased acidity, reduced sugar and phenolic compound content, and lower colour intensity in grapes (García-Escudero et al., 2013).

It is well known that nutrient status in plants is influenced by numerous factors, ranging from complex soil processes (such as pH, carbonates, physical characteristics of the soil, among others), to climatic conditions, and the plant itself (species, age, phenological stage, etc.). In viticulture, this complexity is even more pronounced due to the influence of grape variety, rootstock and clones, as well as the ongoing impact of climate changes. After extensive soil analysis and soil improvement during vineyard establishment, it is advisable in viticulture to perform soil analysis every four years, while foliar analysis should be conducted every season. Foliar analysis is essential for fine-tuning nutrient management since soil status does not fully reflect the actual nutrient composition in the plant, as it is influenced by complex nutrient uptake processes from the soil. Several methods of nutritional plant analysis have been proposed; however, mineral content analysis of leaf blade and petiole are still the most widely used determination methods (García-Escudero et al., 2013; Hickey et al., 2021), while in the past, the entire leaf was analysed (Paprić et al., 2009). In addition, grapevine nutrient guidelines have mostly been developed for two phenological (growth) stages: bloom and veraison (ripening) (AWRI 2010, NSA 2011; Hickey et al., 2021).

Different parts of the leaf contain varying contents of nutrients, and it is important to compare them with appropriate criteria (Melo et al., 2018). When deciding between leaf petiole or leaf blade analysis, there are several advantages and disadvantages to consider. Generally, leaf petiole is easier to sample as a representative sample and has a longer history of application. However, as a transport tissue, it has lower accuracy and exhibits large diurnal and yearly fluctuations in nutrient contents. Additionally, it may fail to diagnose certain nutrient deficiencies, such as Mg, and has a small dynamic range for N. On the other hand, leaf blade analysis provides higher accuracy and wider ranges for nutrient dynamics. However, it requires careful sampling and thorough washing before analysis, as it is prone to sample contamination from dust and chemicals (Hickey et al., 2021). Based on their extensive research, Schreiner and Scagel (2017) recommend using leaf blades, as opposed to petioles, for diagnosing the N, P, and K status of Pinot noir grapevines.

The interpretation of foliar analysis results and the establishment of reference values is a particularly demanding and extensive work, requiring multiyear comprehensive trials. Such research has always been of interest for vineyard management and remains relevant due to ongoing climate and numerous other changes. Extensive studies presenting values by grape varieties, growth periods, various nutrient levels, and the combination of multiple elements are documented in the works of Bergmann and Neubert (1976). Nowadays, several methods have been proposed for interpreting foliar analysis results, including the sufficiency ranges (SR) method, critical values method, DRIS (diagnosis and recommendation integrated system), and DOP (deviation from optimum percentage) (Romero et al., 2014). These methodologies consider different ranges of values and aim to classify each nutrient content as deficient, low, adequate, high or excessive. Establishing these criteria requires extensive surveys of basic data and nutrient contents within a specific region. This process involves the compilation of a comprehensive database over time, which includes various factors such as climate, topography, soil properties, genetics, grapevine varieties and cultivation practices, including irrigation techniques (Romero et al., 2014). Research in Serbia on this topic has experienced periods of vigour in the past, followed by a hiatus. However, there is a renewed focus on these studies, making them highly relevant and deserving of continued attention.

The aim of this study is to highlight the variations in the content of macro and microelements in the soil – grapevine leaf relation in a small-sized vineyard, and additionally, to examine the variations in grapevine leaves based on leaf part and season.

MATERIALS AND METHODS

Location of investigation and sampling

The research was conducted during the vegetation period of 2020 at the Experimental Field of the Faculty of Agriculture, University of Novi Sad in Sremski Karlovci. The observed research plot covers an area of 1.2 hectares planted with the Grašac (Riesling Italico) variety grafted on Keber 5 bb rootstock, clone SK-54. The vineyard was established in 1994, with rows oriented in a north-south direction. The planting distance is 2.8 x 1.6 m and grafts are planted in pair. The vine training system is modified Karlovci training system (one cane and one spur with 12 and 2 buds respectively). The relative elevation difference of the vineyard between the northern highest point bordering the nearby forest and the south-eastern point adjacent to the road is 12 m.

In order to spatially characterize the variability of the soil, the vineyard block was divided into a grid of 20 subplots (Figure 1), each subplot covering an area of approximately 600 m^2 and containing 7 rows. Each individual subplot was then observed as a single sampling location/unit for soil and grapevine leaf. Soil sampling was carried out at three depths (0–30, 30–60, and 60–90 cm) using an agrochemical auger, with one representative sample per subplot consisting of 10–15 individual samples (following the methodology for soil fertility control). This way, a total of 60 soil samples were collected on April 21, 2020.

Figure 1. Layout of divided subplots of observed vineyard block

Grapevine leaf sampling was performed during two phenophases of the grapevine life cycle: flowering (with 80% of plants in this phase) on June 10, 2020, and onset of ripening on August 17, 2020. Sampling was done in the early morning hours, following the methodology for leaf sampling for foliar analysis of macronutrient status. For each grape cluster, an opposite leaf was picked together with its petiole. From each of the 20 subplots, 30 individual leaves were taken evenly as one composite sample. Samples were stored in a paper bag and kept cool during transportation to the laboratory.

Laboratory analysis

The laboratory analyses of collected soil and grape leaf samples were conducted at the Laboratory for Soil and Agroecology of the Institute for Field and Vegetable Crops. The laboratory is accredited according to the SRPS ISO/IEC 17025:2017.

The total of 60 soil samples were air-dried and sieved to a particle size of <2 mm, in accordance with ISO 11464:2006. The pH value in 1:2.5 (v/v) suspension of soil in 1 M KCl was determined upon the ISO method 10390:2005. The free $CaCO_3$ content was determined by volumetric method ISO 10693:1995. Organic matter content was measured by sulfochromic oxidation method (Tyurin's method). Total organic carbon (TOC) and total nitrogen were determined by elementary analysis (CHNSO VarioEL III) after dry combustion in accordance with the ISO 10694:1995 and AOAC Official Method 972.43:2006, respectively. Readily available phosphorus and potassium were extracted by ammonium lactate extraction, and measured by the means of spectrophotometry and flame photometry, respectively (Egnér et al., 1960). Particle size distribution was determined in the <2 mm fraction by the pipette method (Van Reeuwijk, 2002). The size fractions were defined as clay (<2 µm), silt (2–20 µm), fine sand (20–200 µm) and coarse sand (200–2,000 µm). Cation exchange capacity (CEC) was determined by ammonium acetate (Chapman, 1965). The content of available microelements was determined by soil extraction using DTPA according to the ISO 14870:2001 method. The determination of available Boron content was performed after soil extraction in hot water. The detection of Cu, Zn, Fe, Mn and B from prepared solutions was carried out using the ICP-OES Vista Pro Axial instrument, Varian, following the US EPA 200.7:2001 method.

For the purpose of foliar analysis, the collected leaf samples were thoroughly rinsed with distilled water and air-dried. Subsequently, the leaf petiole was separated from the leaf blade. In this manner, a total of 80 individual plant material samples were analysed: 20 samples from each observed subplot at two time intervals, consisting of both leaf blade and leaf petiole for each sample. The samples were ground using a plant material mill. Prior to further sample analysis, the moisture content was determined gravimetrically and the presented results are based on dry weight. Total nitrogen was analysed using the elemental analysis on the CHNSO VarioEl. III following the AOAC 972.43:2006 method. The determination of P, K, Mg, Ca, B, Mn and Zn content was conducted after microwave wet digestion of the samples in a mixture of $ccHNO_3$ and H_2O_2 with gradual heating up to 180 °C using the Milestone ETHOS1 instrument with a digestion time of 35 minutes. The detection of elements was performed using the ICP-OES Vista Pro Axial instrument, Varian, following the US EPA 200.7:2001 method.

Statistical data processing

Data were statistically processed by analysis of the main descriptive parameters and correlations between examined parameters. All statistical analyses were performed using STATISTICA for Windows version 13 (TIBCO, 2018). Statistical parameters were shown in tables 1 and 5.

RESULTS AND DISCUSSION

Soil properties and variability

According to the analysis of physical soil properties, the particle size distribution indicates that the observed area primarily falls into two texture classes clay loam and light clay based on the IUSS classification. However, there is one sample from the higher part of the plot (sample ordinal number 18) (Figure 1) that belongs to the class of rough sandy loam (30–60 cm), characterized by a high sand content. On observed plot, in the upper soil layer (0–30 cm), the texture classes of clay loam and light clay are relatively equally distributed, while in deeper soil layers, the dominant texture class is light clay. Based on the conducted descriptive statistics (Table 1), the greatest variation in data is observed in the soil layer at 30–60 cm depth. Overall, the largest differences were found in the sand content, primarily due to the presence of one sample (No. 18) with a high sand content of 89.52%.

According to the determined pH values, the tested samples range from 7.08 to 7.79, indicating a variation from neutral to slightly alkaline soil class (Table 1). Based on the average pH values, the pH reaction decreases with depth, and in terms of spatial distribution, the eastern and southern edges of the plot exhibit higher pH values. The content of free carbonates expressed as $CaCO_3$, according to the average values, exceeds the threshold for highly carbonate soils (>10%) and remains relatively consistent with depth (Table 1). However, individual samples exhibit a wide range of variation, encompassing all classes from non-carbonate to highly carbonate soils. The carbonate content shows the least variation in the surface layer of the soil, while the variability among samples increases with depth. The spatial distribution of carbonate content aligns with the pH distribution. The variability of Cation exchange capacity (CEC) is relatively consistent within each soil layer (Table 1). Generally, higher CEC values were observed in the western part of the plot. The organic matter (humus) content is very low in all tested samples, falling within the class of very low to low humus soil. As expected, it decreases with depth, with the highest value recorded as 1.53 in the upper soil layer (Table 1). The western and upper parts of the plot exhibit the lowest humus content.

Table 1. Descriptive statistics of soil properties at three depths (20 subplots of 1.2 hectare)

Parameter	Depth [cm]	Average	Min.	Max.	Percentile 25	Percentile 75	Variance	Std. dev.*
Sand [%]	0–30	47.59	37.61	70.45	42.84	50.89	39.63	8.27
	30–60	46.46	33.02	89.52	40.36	46.95	101.94	12.84
	60–90	44.48	33.11	65.53	39.39	47.39	33.28	7.86
Silt [%]	0–30	27.88	20.32	33.40	26.32	28.64	8.28	2.88
	30–60	28.10	12.20	36.60	26.54	31.12	25.29	5.03
	60–90	29.07	21.88	35.60	26.98	30.30	13.00	3.61

Clay [%]	0–30	24.53	17.64	29.00	22.88	26.90	9.39	3.06
	30–60	25.44	12.88	30.24	23.46	28.36	19.07	4.37
	60–90	26.45	18.48	34.04	24.38	29.80	17.79	4.22
pH in KCl	0–30	7.43	7.24	7.67	7.35	7.52	0.01	0.12
	30–60	7.40	7.14	7.79	7.26	7.54	0.03	0.17
	60–90	7.38	7.08	7.77	7.26	7.54	0.04	0.19
CEC [meq/100g]	0–30	16.81	12.27	19.35	15.77	18.49	4.54	2.13
	30–60	17.24	11.85	21.13	15.10	19.15	7.65	2.77
	60–90	17.63	12.42	21.49	15.99	19.27	6.59	2.57
$CaCO_3$ [%]	0–30	11.09	4.20	21.46	6.52	14.30	22.89	4.78
	30–60	11.15	4.21	29.45	6.31	11.78	61.66	7.85
	60–90	11.47	1.40	31.13	6.31	13.04	59.30	7.70
OM [%]	0–30	1.15	0.75	1.53	0.98	1.30	0.05	0.22
	30–60	0.82	0.25	1.40	0.63	0.98	0.08	0.28
	60–90	0.66	0.13	1.12	0.44	0.83	0.08	0.27
P_2O_5 [mg/100g]	0–30	18.27	6.44	28.48	12.28	24.41	51.19	7.15
	30–60	7.64	1.81	13.70	4.84	10.78	11.04	3.32
	60–90	5.16	2.46	14.26	3.54	5.55	8.46	2.91
K_2O [mg/100g]	0–30	18.79	8.50	28.36	17.04	21.43	21.40	4.63
	30–60	12.22	4.59	18.72	11.20	13.49	10.15	3.19
	60–90	11.48	5.02	29.95	9.17	12.56	26.05	5.10
Cu-DTPA [mg/kg]	0–30	7.38	2.36	21.64	5.43	7.98	15.44	3.93
	30–60	2.77	0.61	6.57	1.47	3.71	2.66	1.63
	60–90	1.41	0.33	3.76	0.84	1.72	0.76	0.87
Fe-DTPA [mg/kg]	0–30	9.91	6.27	16.20	8.34	10.63	5.59	2.36
	30–60	11.09	5.17	18.05	9.42	13.10	10.39	3.22
	60–90	12.33	6.46	21.86	10.43	13.77	15.36	3.92
Mn-DTPA [mg/kg]	0–30	6.22	4.99	7.98	5.34	7.22	1.13	1.06
	30–60	6.95	5.37	9.21	5.79	8.27	1.75	1.32
	60–90	7.80	5.28	13.32	6.79	8.68	3.09	1.76
Zn-DTPA [mg/kg]	0–30	2.16	0.95	9.11	1.48	2.31	2.88	1.70
	30–60	0.80	0.21	1.65	0.62	0.97	0.13	0.36
	60–90	0.64	0.24	1.43	0.37	0.90	0.11	0.33
B-H_2O [mg/kg]	0–30	0.37	0.14	1.04	0.25	0.42	0.04	0.19
	30–60	0.28	0.08	0.72	0.20	0.33	0.02	0.13
	60–90	0.26	0.06	0.51	0.18	0.35	0.01	0.11

[*] Standard deviation

The content of readily available phosphorus, expressed as P_2O_5, shows significant variation in the upper soil layer (Table 1). In the soil layer at 30–60 cm, where the root activity of grapevines is highest, phosphorus levels are generally low. The optimal phosphorus content was determined in only four out of the 60 tested samples. Half of the samples fall into the class of very low and low content, while one-fifth (12 samples) belong to the class of medium content. Additionally, nine samples exhibit high phosphorus content, all of which originate from the surface soil layer. In general, higher phosphorus content is found in the lower parts of the observed plot. Based on the average value of available potassium content, expressed as K_2O, the soil is classified as low to medium in terms of potassium content. Regarding the soil depths, the potassium content shows the highest variation in the surface (0–30 cm) and deepest layers (60–90 cm), ranging from very low to optimal classes. There are no excesses of potassium observed. In the soil layer at 30–60 cm, where the root activity of grapevines is highest, none of the samples reach the optimal level. Out of the 20 observed samples in the 30–60 cm soil layer, 16 belong to the low content class (7–15 mg / 100g), while two samples fall into the very low (<7 mg / 100g) and medium content (15–20 mg / 100g) classes, respectively. Similar to the spatial distribution of phosphorus, potassium content is higher in the lower parts of the observed plot.

Regarding accessible microelements in the soil, copper deficiency is not expected in vineyards due to the intensive use of copper-based fungicides. The copper content is the highest in the surface layer of the soil, where the highest variance and standard deviation of results are present as well, indicating its anthropogenic origin and it decreases with depth. The observed soil is generally adequately supplied with copper in deeper layers (Table 1 and Table 2). However, in two specific parts of the vineyard (sample No. 4 and No. 17), the copper content in the surface layer reaches notably high contents of 21.64 and 10.84 mg/kg, respectively. In contrast, other areas of the vineyard's surface layer have copper contents below 10 mg/kg. Content above 10 mg/kg suggest an anthropogenic origin and may potentially have phytotoxic effects on the vigour of young plants.

The observed soil is generally well supplied with available iron, and its content is consistent throughout the soil profile, with all recorded contents above the minimum values (Table 1 and Table 2). The lowest iron content was found in the parts of the vineyard covered by samples No. 12, 18 and 19. The iron content varies the most in the deepest layer of the soil. The content of available manganese is the most consistent among all observed elements, indicating that the soil is generally well supplied with this micronutrient values (Table 1 and Table 2).

The content of available zinc generally decreases with depth and is at a low level values (Table 1 and Table 2). Below the minimum threshold of 0.6 mg/kg, there are 15 samples in the deeper soil layers, specifically four sections of the vineyard located in the 30–60 cm soil layer (samples No. 9, 16, 18, and 19). The determined zinc contents highlight the necessity of implementing additional zinc fertilization to address the observed deficiency.

The content of available boron is very low. Out of the 60 analysed samples, 42 have values below the minimum threshold of 0.35 mg/kg (Table 2). In the 30–60 cm soil layer, where the root activity of grapevines is highest, only four sections of the vineyard have boron content exceeding 0.3 mg/kg (samples No. 12, 12, 13 and 17). When applying boron fertilizers to the soil, extreme caution should be exercised. It is necessary to determine the precise amount of fertilizer, as there is a fine line between optimal quantities (1.5–2.0 mg/kg) and amounts that can have toxic effects (5 mg/kg) (Ubavić et al., 2008).

Table 2. Minimum and optimal values of microelements in the soil for grapevines (Lanyon et al., 2004; Ubavić et al., 2008; Ninkov et al., 2019)

Assessment of the level of provision	Cu	Fe	Mn	Zn	B
			[mg/kg]		
Minimum values	0.2	2.5–4.5	2.0	0.6	0.35
Optimal values	1.2–2.4	11–21	10–20	3–6	1.5–2.0

Overall, the tested 20 subplots exhibit variation in the physico-chemical characteristics of the soil, with the highest variability observed in the 30–60 cm soil layer, where grapevine root activity is the greatest. The soil generally exhibits low levels of organic matter, macronutrients such as phosphorus and potassium, as well as micronutrients like zinc and boron. The spatial distribution of the physicochemical parameters of the soil indicates the presence of erosion processes at the investigated microlocation.

Foliar analysis, content and seasonal dynamics of nutrients

Based on the analysis of two parts of grapevine leaves (blade and petiole) during two phenophases (flowering and ripening), differences in nutrient content have been observed both among leaf parts and across seasons (Table 3). Nitrogen, boron and manganese are found in higher contents in the leaf blade, specifically during the flowering (sampling in June), compared to the second sampling in August during the ripening. Phosphorus and potassium are found in higher contents in the leaf petiole, while the content of P remains relatively consistent across the two seasons. The content of K is higher in the petiole during the flowering phase (sampling in June). It has been determined that there are higher levels of magnesium and calcium in the leaf petiole, particularly during the ripening phase in August (Table 3). Finally, zinc shows seasonal variation, with higher contents observed in the leaf blade during June, while higher contents are found in the leaf petiole during August (Table 3). These findings are broadly consistent with previous research and the established nutrient dynamics, where the content of N and K is highest in leaves during spring and decreases throughout the season. The content of P in leaves is highest before flowering, and additionally, the content of Ca and Mg is higher in older leaves (Burić, 1979).

Table 3. Nutrients content in grape leaves (mean value) according to leaf part and sampling time

Leaf part	Sampling time	N	P	K	Mg	Ca	B	Mn	Zn
				[%]				[mg/kg]	
Blade	June	2.77	0.21	0.62	0.35	2.48	35.50	218.93	32.79
	St. Dev.	0.11	0.03	0.04	0.03	0.20	7.86	35.19	5.89
	August	2.16	0.17	0.40	0.46	3.42	33.28	209.31	28.47
	St. Dev.	0.09	0.02	0.03	0.06	0.18	7.44	32.32	3.28
Petiole	June	0.84	0.28	0.78	0.81	2.07	24.39	63.17	26.82
	St. Dev.	0.08	0.08	0.14	0.08	0.19	3.13	15.15	8.18
	August	0.64	0.24	0.57	1.69	3.30	29.05	205.92	42.16
	St. Dev.	0.08	0.12	0.22	0.19	0.30	1.76	86.74	15.71

According to literature (Goldspink et al., 2000; Verdenal et al., 2021) N content in the leaf blade is very different to that in the petiole: petiole N content is more sensitive to variations in N nutrition than leaf blade N content, which is more constant. Consequently, the chosen analysis (i.e., on either the leaf blade or petiole, or both together) will greatly affect the results and require adapted interpretation thresholds (Verdenal et al., 2021).

In European vineyards, leaf blades are the standard tissue for diagnosing vine nutrients (OIV, 1996). On the other hand, the petioles are the tissue of choice for routine diagnosis of vineyard nutrient status in United States and Australia (Robinson et al., 1978; Christensen, 1984). Benito et al. (2013) suggest that diagnosis of nitrogen, phosphorus, potassium, manganese and zinc is preferable in the blade at complete cap-fall, fruitset and veraison, while the petiole is a better choice for iron and boron at both complete cap-fall and fruitset, and for boron at veraison. Calcium and magnesium are likely to be found at flowering or veraison, and iron at veraison, independently of sampling tissues.

Table 4 presents optimal nutrient content thresholds in grapevine leaves based on various literature sources, illustrating variations influenced by factors such as authorship, season, leaf segment, and research location. It is important to note that Table 4 offers a foundational overview. However, there are additional studies establishing optimal values, deficiencies, excesses, etc. Furthermore, some studies even provide insights into grapevine varieties and specific rootstocks. The production guidelines, based on ranges of nutrient status from the literature, are indicative for grape producers and testing laboratories (Schreiner and Scagel, 2017). The benefit for them will be if production guidelines take into account the effects of nutrients on vine productivity and must quality.

Comparing the outcomes of our spring foliar analysis (Table 3) with the reference values detailed in Table 4 (despite their variations attributed to different authors), it is deduced that the content of N and P in grapevine leaves is at the lower optimal threshold. Potassium content is notably deficient, in both the blade and petiole. In contrast, Mg and Ca contents are satisfactory, with Ca even reaching the upper limit of sufficiency. Distinct thresholds for Mn and Zn are presented, placing their content within the upper sufficiency optimal limit and well within the sufficiency range, respectively. Despite these distinct threshold levels, the content of B is notably deficient, particularly in the petiole.

The stated findings based on foliar analysis of deficiencies in certain elements are in complete alignment with soil analyses, except in the case of Zn. Soil analysis revealed low levels of organic matter, P, K, and B. The Zn content in grapevine leaves is sufficient, while specific parts of the observed plot are characterized by low levels of this element.

It is important to emphasize that a synchronized fertilization recommendation can be deduced from both soil and foliar analyses, especially concerning nutrients N, K and B. Nonetheless, according to soil analysis, a slightly elevated application of P and zinc Zn could be contemplated within the scope of fertilizer management. Importantly, with leaf P content at the lower threshold and Zn content well within the optimal range, increased quantities of P and Zn would not adversely affect the vineyard's ecosystem.

Based on the results of the entire study, correlations between all observed parameters were determined. The strongest correlations were obtained between soil parameters from all three depths and nutrients in the leaf blade sampled in flowering phase (Table 5).

The nutrient content in the soil, specifically nitrogen, potassium, manganese, and zinc, showed a positive statistically significant correlation with the respective nutrient content in the leaf blade. However, this relationship was not observed for phosphorus and boron.

The soil pH and carbonate content exhibited a negative and statistically significant correlation with the content of P, K and B in the leaf blade. The influence of high soil carbonate content, and consequently elevated pH levels, on nutrient uptake inhibition has been the subject of thorough investigation (Cambrollé et al., 2015). On the other hand, soil carbonate showed a positive correlation with the content of Mg and Mn in the grapevine leaf blade. However, the negative correlation between carbonate content in soil and Ca uptake and is unexpected. On the other hand, soil available phosphorus showed a statistically significant positive correlation only with the Ca content in the leaf blade. The complex process of calcium uptake, involving various transport pathways, remains a subject of ongoing debate in research studies (Duan et al., 2022; Nistor et al., 2022) and influenced by a many of factors, including water stress. It is widely acknowledged that mature leaves typically exhibit elevated calcium content what is also found in present study (Table 3).

Table 4. Optimal values for the interpretation of grapevine leaf nutrient content according to literature, on dray mass

	Source	Location	Leaf part	Season		N	P	K	Mg	Ca	B	Mn	Zn	Fe
						[%]					[mg/kg]			
1	Bergmann, 1986		whole leaf	flowering	LL	2.30	0.25	1.20	0.25	1.5	30	30	20	
					UL	2.80	0.45	1.60	0.60	2.5	60	100	70	
2	Paprić et al., 2009		whole leaf		LL	2.50	0.22	1.3	0.25					
					UL	2.75	0.24	1.4						
3	Melo et al., 2018	Brazil	whole leaf		LL	2.40	0.29	1.1	0.26	1.2	26	390	150	89
					UL	3.00	0.39	1.4	0.33	1.6	39	578	256	140
4	Verdenal et al., 2021		whole leaf		LL	2.00								
					UL	2.30								
5	AWRI, 2010	Australia	blade	flowering	LL	3.00	0.25	1.0	0.3	1.2	30	25	30	
					UL	5.00	0.40	1.8	0.6	2.8	200	200	60	
6	García-Escudero et al., 2013	Spain	blade	flowering	LL	3.13	0.27	0.9	0.3	2.1	58	68	18	105
					UL	3.28	0.31	1.0	0.4	2.3	67	87	20	131
7	Verdenal et al., 2021		petiole		LL	0.40								
					UL	0.60								
8	AWRI, 2010	Australia	petiole		LL	0.80	0.25	1.8	>0.4	1.2	35	30	>26	>30
					UL	1.10	0.50	3.0		2.5	70	60		
9	NSA, 2011	Canada	petiole	flowering	LL	1.60	0.16	1.5	0.2	0.4	25	20	20	40
					UL	2.50	0.60	4.0	0.4	1.5	50	150	100	180
10	García-Escudero et al., 2013	Spain	petiole	flowering	LL	0.94	0.27	1.3	0.6	1.4	40	23	14	22
					UL	1.10	0.34	1.8	0.7	1.5	42	29	17	25
11	Goldspink et al., 2000	Australia	petiole	flowering	LL	0.9	0.30	1.3	>0.4	1.2	30	25	15	
					UL	1.2	0.49	3.0		2.5	70	500	25	

LL = lower limit of optimal content
UL = upper limit of optimal content

Table 5. Correlation coefficients between soil parameters at three depths and leaf nutrient status of leaf blade sampled in June (flowering)

	Leaf blade, flowering							
Soil	N	P	K	Mg	Ca	B	Mn	Zn
pH-KCl	-0.162	-0.421*	0.032	0.326*	-0.407*	-0.556*	0.372*	-0.230
$CaCO_3$	-0.236	-0.522*	-0.173	0.315*	-0.460*	-0.491*	0.562*	-0.026
OM	0.271*	0.444*	0.316*	-0.132	0.485*	0.471*	-0.272*	-0.094
TOC	-0.058	-0.018	0.116	0.283*	0.033	-0.004	0.178	-0.108
N-Total	0.310*	0.437*	0.259*	-0.088	0.487*	0.518*	-0.282*	-0.072
P_2O_5	0.174	0.215	0.201	0.029	0.293*	0.251	-0.155	-0.100
K_2O	0.262*	0.292*	0.335*	-0.047	0.386*	0.278*	-0.243	-0.070
Coarse sand	-0.156	-0.273*	-0.040	0.058	-0.281*	-0.466*	0.085	-0.027

Fine sand	0.433*	0.333*	0.197	0.167	0.358*	0.501*	-0.210	-0.308*
Silt	-0.121	-0.261*	-0.162	0.075	-0.168	-0.094	0.370*	0.148
Clay	0.073	0.496*	0.085	-0.303*	0.398*	0.547*	-0.362*	0.132
CEC	0.227	0.542*	0.023	-0.322*	0.480*	0.688*	-0.406*	0.168
Cu-DTPA	0.201	0.195	0.235	-0.024	0.272*	0.141	-0.101	-0.077
Fe-DTPA	-0.073	0.031	-0.090	-0.421*	0.007	0.039	0.172	0.287*
Mn-DTPA	-0.195	-0.267*	-0.276*	-0.347*	-0.264*	-0.304*	0.472*	0.335*
Zn-DTPA	0.003	-0.006	0.058	0.017	0.195	0.040	0.116	0.287*
B-H_2O	0.060	-0.124	-0.042	-0.024	0.123	0.160	-0.029	0.094

* Significantly correlated p=0.95

Soil organic matter, total nitrogen, and available potassium content are significantly positively correlated with the major nutrients in the leaf blade: N, P, K, as well as with the content of B and Ca, which indicates importance of applying good fertilizer management. Total nitrogen is negatively correlated with the content of Mn, while total organic carbon is positively correlated only with the content of Mg in the leaf blade. The cation exchange capacity (CEC) of the soil showed a statistically significant positive correlation with the content P, Ca and B in the leaf blade. However, CEC exhibited a negative correlation with the content of Mg and Mn in the leaf blade. Stated findings indicated antagonistic interactions between elements. The influence of soil particle distribution on nutrient content has been determined, particularly on the phosphorus content in the leaf blade. However, the obtained correlations are not logical for drawing a general conclusion.

CONCLUSION

The tested 20 subplots of the small sized plot of 1.2 ha exhibit variation in the physico-chemical characteristics of the soil, with the highest variability observed in the 30–60 cm soil layer. The soil generally exhibits high pH reaction, low levels of organic matter, available P, K, as well as low levels of micronutrients Zn and B. The spatial distribution of the physico-chemical parameters of the soil indicates the presence of erosion processes at the investigated microlocation.

Based on the analysis of two parts of grapevine leaves (blade and petiole) during two phenophases (flowering and ripening), differences in nutrient content have been observed among leaf parts and across seasons, which broadly aligns with established nutrient dynamics described in the literature. Based on foliar analysis and by comparing nutrient levels in the leaves during the flowering phase with various literature sources' threshold values, it was found that the content of N and P is at the lower limit, while the deficiency of K and B is clearly confirmed. Synchronized fertilization recommendations can be inferred from both soil and foliar analyses, particularly for nutrients N, K, and B. However, based on soil analysis, a slightly increased application of P and Zn would be recommended for fertilization.

Based on the established correlations among all observed variables, the highest correlations were determined between soil parameters across all three depths and nutrients in the leaf blade sampled during the flowering phase. The nutrient content in the soil, specifically N, K, Mn, and Zn, showed a positive statistically significant correlation with the respective nutrient content in the leaf blade. However, this relationship was not observed for P and B. The identified correlations indicate the influence of pH and carbonates on reduced uptake of certain elements, as well as the presence of nutrient uptake antagonism in the grape leaves depending on the physicochemical soil parameters.

Further research is necessary to lay the foundation for the development of accurate and reliable criteria for diagnosing nutrition, not only for the whole species but also among grapevine leading varieties. Given the significant variations in nutrient requirements and accumulation among these genotypes, this research will be instrumental in ensuring optimal nutrient supply while minimizing deficiencies or excesses.

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ОРИГИНАЛНИ РАД

ОДНОС САДРЖАЈА МИКРО И МАКРОЕЛЕМЕНАТА У ЗЕМЉИШТУ И ЛИСТОВИМА НА МИКРОЛОКАЦИЈИ ВИНОГРАДА

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РЕЗИМЕ: Спроведено истраживање има за циљ да утврди варијације у садржају макро- и микроелемената у односу земљиште–лист у малим виноградима. Парцела величине 1,2 хектара у Сремским Карловцима (Србија), са сортом *грашац* (Riesling Italico), подељена је на 20 делова, под парцела. Свака под парцела служила је као појединачна локација за узорковање земљишта и листова. Узорци земљишта су сакупљани на три дубине, док је узорковање листова обављено у две фенофазе (фаза цветања и фаза сазревања), са раздвајањем дршке и лиске. Варијације у физичко-хемијским карактеристикама земљишта су потврђене међу посматраних 20 под парцела, са највећом варијабилношћу у слоју земљишта од 30–60 cm. Земљиште генерално показује низак садржај органске материје и приступачног фосфора (P), калијума (K), цинка (Zn) и бора (B). Просторна дистрибуција физичко-хемијских параметара указује на присутне процесе ерозије. Добијене разлике у садржају хранљивих материја међу деловима листа и фенофазама су у складу са постојећом литературом. При упоређивању садржаја хранљивих материја у листовима са оптималним вредностима из литературе, утврђено је да је садржај азота (N) и фосфора (P) на доњој граници, и потврђен је недостатак калијума (K) и бора (B). Идентичне препоруке за ђубрење могу се донети на основу анализе земљишта и фолијарне анализе, пре свега за азот (N), калијум (K) и бор (B). Додатно, на основу анализе земљишта, препоручује се благо повећање примене фосфора (P) и цинка (Zn) за ђубрење. Утврђене корелације између свих посматраних променљивих откриле су везе између параметара земљишта на све три дубине и хранљивих материја у листу током фазе цветања. Садржај хранљивих материја у земљишту, нарочито азота (N), калијума (K), мангана (Mn) и цинка (Zn), показује статистички значајне позитивне корелације са садржајем одговарајућег елемента у листу током фазе цветања. Неопходна су даља истраживања како би се поставили темељи за развој тачних и поузданих критеријума за дијагнозу исхране винове лозе, не само за целу врсту него и међу њеним водећим сортама. С обзиром на значајне варијације у захтевима за хранљивим материјама и акумулацији међу овим генотиповима, оваква истраживања би била од кључног значаја за достизање оптималног снабдевања винове лозе хранљивим материјама, избегавајући њихов недостатак или сувишак.

КЉУЧНЕ РЕЧИ: земљиште винограда, фолијарна анализа, винска сорта *грашац*

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ORIGINAL ARTICLE

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THE IMPACT OF GLOBAL WARMING ON MODERN OENOLOGICAL PRACTICE

ABSTRACT: A number of publications deal with the influence of global warming on the chemical composition and sensory properties of grapes and wines. High-temperature values shorten the time needed for grape harvesting. All parts of the berries cannot reach full ripeness and the sugar concentration in the grapes increases. The acidity of the grapes often decreases, which is accompanied by an undesirable increase in pH. All this leads to harvesting the grapes before the berries have reached their full technological maturity, which refers mainly to the skin and the seeds. The insufficient ripeness of the grapes affects the chemical composition and the quality of the wine. The wine does not have the required color intensity. With a long maceration, there is a risk of increased extraction of bitter tannins. In this situation, oenologists must apply the technological procedure used in conditions of incompletely ripened grapes, or wait for the grapes to fully ripen and then apply technological procedures to reduce alcohol content and pH. Thus, the variability of the climate from year to year affects the quality of the grapes and consequently the quality of the wine.

KEYWORDS**:** global warming, phenolic maturity, wine

INTRODUCTION

It is well known that the quality of wine depends primarily on the variety or type of vine, but also on the environmental conditions in which it is grown. Hence the saying that the vine is the mother of wine, the soil is the father and the climate is destiny. Thus, the variations in the climate from year to year lead to changes in the quality of the grapes and consequently, in the quality of the wine. Optimal conditions for growing vines, especially for producing high-quality wines, are given when the following climatic factors are harmonized in their influence: sunlight, temperature, precipitation and wind. The vine is very demanding as far as the temperature is concerned. The growing period of the vine is relatively long, ranging from 175 to 245 days. The vine

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is also very demanding in terms of temperature during the growing season, as the yield and quality of the grapes and wine depend on it as well as on well-distributed rainfall. The successful cultivation of this crop requires at least 1,350 hours of sunshine per year and an average annual temperature of 9–10 °C. However, when it comes to temperature, one should be careful, because the rule that the more sun – the better the wine is contradicted by the fact that the best wine in France is not in the south, where the average annual temperature is 14 °C, but in the Loire Valley and Bordeaux, where the average temperatures are 12 °C and 12.5 °C. The best Italian wines are also not being produced in the south of Italy, Sicily, especially the white wines. It is widely known that under the southern sun of the Mediterranean or California, wines gain in weight, i.e. richness, alcohol and color, but on the other hand they lose piquant acids and aromatic compounds. It is obvious that at the expense of fullness of flavor, typicality is lost, i.e. the distinct varietal specificity with prominent pleasant acids. In addition to a certain temperature regime, the quality of grapes and wine is also influenced by the water regime of the soil, which depends on the amount of precipitation during the growing season. An annual rainfall of 600 mm to 800 mm is considered optimal, although it should be emphasized that on some soils good grape yields and wine quality are obtained with a rainfall of 300 mm (Milosavljević & Jović, 1999).

Global changes require new strategies in grape and wine production

For the last twenty years, there has been talk about global warming and the problems it could cause in various areas of human life and activity. About ten years ago, numerous papers were published dealing with the effects of global warming on the chemical composition and sensory properties of grapes and wine, as well as the search for methods to remedy the negative effects on wine quality. Nowadays, it is known that the use of fossil fuels leads to an increase in CO_2 and other gases responsible for the occurrence of the greenhouse effect and the warming of the planet Earth. The gases present in the atmosphere absorb thermal infrared rays (IR). Without these gases intercepting the IR rays, the temperature of the Earth would be -18 °C and not +15 °C. The increase in CO_2 emissions is the main reason for this. For example, the concentration of CO_2 at the beginning of the 20th century was 260 ppm, today it is 380 ppm, and by the end of the 21st century, it is expected to reach 450 to 1,000 ppm. The greater retention of IR rays has led to an increase in the Earth's temperature by 0.5 and in some parts of the world by as much as 2 °C. Some studies have shown that if this trend of global warming continues, the temperature in France, for example, will increase by 6 °C by the end of this century (Seguin & Gaudillère, 2007). The increase in temperature as a result of the above changes is directly reflected in the earlier flowering of the vines, resulting in an earlier harvest. In the last 50 years, the harvest has moved forward by 20 to 30 days worldwide. In Serbia, harvesting is increasingly taking place in the month of August, when not all parts of the grape have reached the required maturity. The higher CO_2 emission directly affects viticultural production. Photosynthesis is accelerated by 20% to 30%, increasing biomass by 15 % to 20%. Accelerated photosynthesis results in greater leaf mass and thus faster accumulation of sugars. The increase in biomass requires 10% more water, which will require mandatory irrigation in the future. The influence of climate change on the ripening of grapes is obvious and has a strong impact on the chemical composition and quality of wine. As global warming has a strong impact on the increasing imbalance between primary and secondary metabolites in grapes, the question for modern oenology is what processes and measures can be used to mitigate this imbalance. As temperatures rise above normal, high concentrations of sugars accumulate in grapes, often causing acidity to drop significantly with an undesirable increase in pH. These circumstances lead to grapes being harvested before the berry has reached full ripeness, which refers mainly to the skin and seeds. The berry in this apparent full ripeness has a significant impact on the chemical composition and quality of the wine.

Conditions of high temperatures during grape ripening result in rapid accumulation of sugars in grapes and faster degradation of organic acids. Global warming is directly reflected in the rapid accumulation of sugars in grapes, resulting in wines with a high alcohol content (an increase of about 2% v/v over the last 20 years), a decrease in total acidity of 1 g/l (expressed in wine) and an increase in pH of 0.2 units (Van Leeuwen & Darriet, 2016). This leads to the formation of high-potential alcohol and a significant increase in pH in a shorter time, which directly affects the earlier harvest. This is particularly unfavorable for winemaking in white varieties. The high alcohol content inhibits the development of the aromatic complex of wine due to interactions with aromatic compounds (Dequin, 2007). There is a tone of distillate on the odor (burnt tone) and dryness in the aftertaste (similar to bitterness). On the other hand, low acidity makes wines with high alcohol content “dull” and “heavy” in taste (Petrović, 2022). High pH levels allow the development of microorganisms that cause spoilage of foods and beverages (acidification of foods is a traditional method of their preservation). For example, beer has a higher pH than wine ($pH > 4$), so it is attacked by enterobacteria, bacilli, micrococci, staphylococci, various lactic acid bacteria and spoilage yeasts, which is also the case with wine. However, compared to beer, wine is better protected due to its higher alcohol content. As already emphasized, in the production of red wines, the degree of ripeness of the skins, especially the seeds, which are often not yet ripe at the time of harvest, has a very important, if not decisive, influence on the quality of wine. For all these reasons, the abovementioned climatic changes affect the ever-increasing imbalance between technological (industrial) and phenolic maturity. Figure 1 shows the impact of global warming on the grape ripening process and the imbalance between technological (industrial) and phenolic maturity (Zamora, 2007).

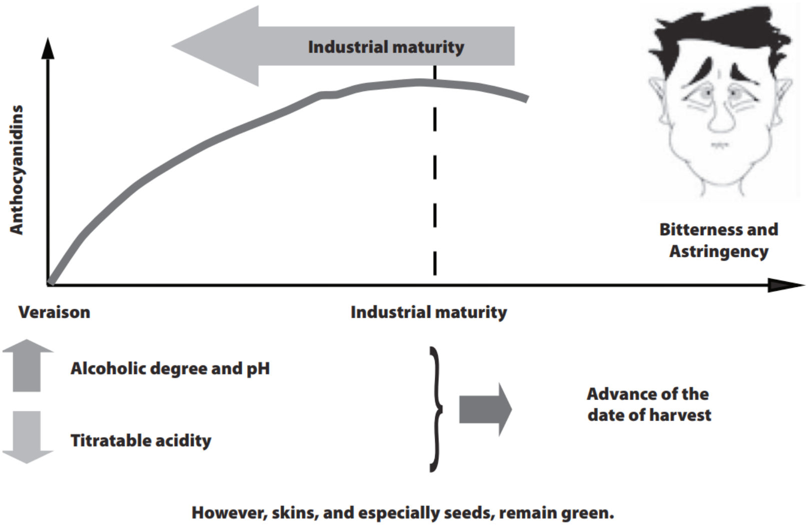

Figure 1. Effects of global warming on the increasing imbalance between industrial and phenolic ripeness (Zamora, 2007)

To better understand the impact of these phenomena on red wine quality, it is necessary to analyze the kinetics of dissolution (extraction) of phenolic compounds during winemaking. As shown in Figure 2, the color intensity and concentration of anthocyanins increase until they reach a peak, and then slowly decrease. Skin tannins are dissolved immediately after the maceration of the grapes in the pre-fermentative phase, and their concentration continues to increase throughout the maceration process. In contrast, the dissolution of tannins from the seeds does not start at the beginning of maceration, since their extraction requires the presence of ethanol and a slightly higher temperature (Zamora, 2007).

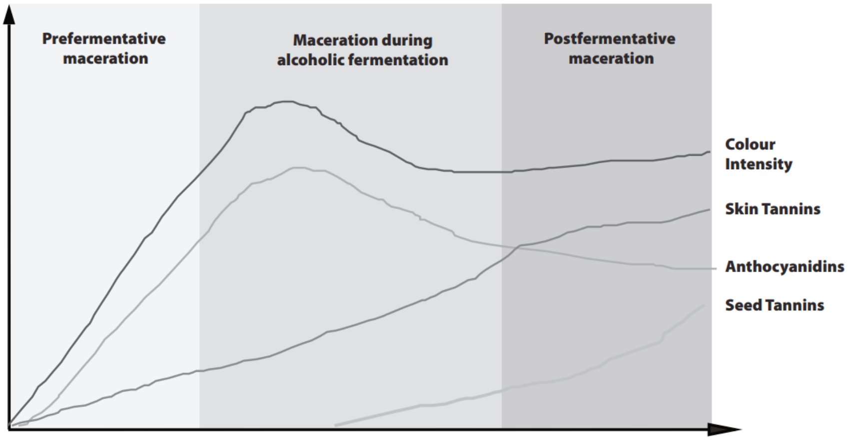

Figure 2. Extraction kinetics of phenolic compounds throughout the maceration/fermentation process (Zamora, 2007)

Considering the dynamics of phenolic compounds content during grape ripening (Figure 3), the development of pungency is different in all parts of the berry. After the vèraison, the content of colored compounds (anthocyanins) and tannins in the berry skin increases to a maximum. After that, their content does not change, or the concentration decreases slightly. In contrast, the concentration of tannins in the seeds decreases throughout the ripening period of the berries. This could be an explanation why wines from unripe grapes contain many astringent tannins from the seeds and relatively few tannins from the berry skin.

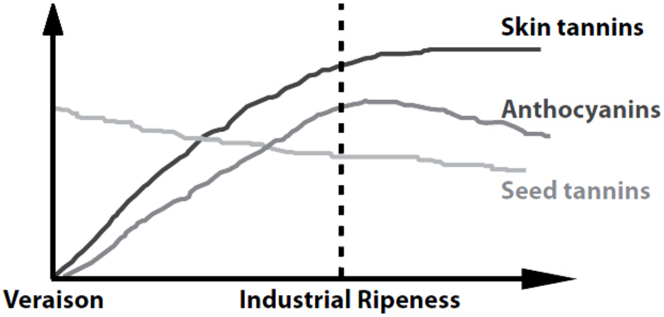

Figure 3. Evolution of phenolic compounds throughout the ripeness process (Zamora, 2007)

The reason why seed tannins are stronger and rougher than skin tannins is due to their chemical composition. In both cases, they are condensed tannins, also called proanthocyanidins, which are polymers of the monomeric subunits shown in Figure 4: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin gallate.

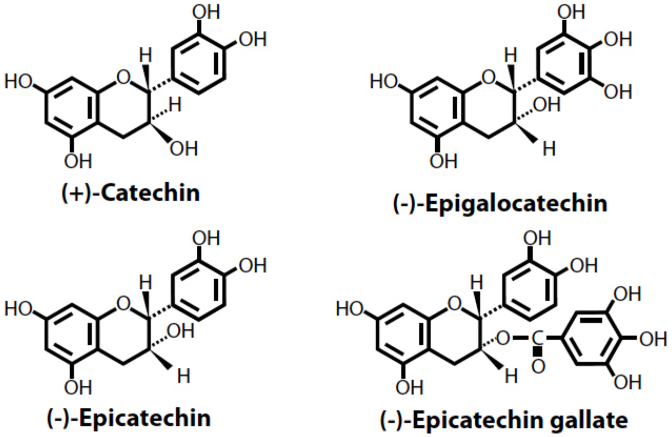

Figure 4. Structure of monomeric tannin units (Zamora, 2007)

The degree of astringency, i.e., pungency of proanthocyanidins is determined by their molecular weight and the proportion of epicatechin gallate. If the degree of polymerization is higher with a higher proportion of epicatechin gallate, the pungency is more intense. Research has shown that skin tannins are characterized by a higher molecular weight and the presence of epigallocatechin, while seed tannins contain a higher proportion of epicatechin gallate, which is the cause of their greater pungency and bitterness. Figure 5 shows the different degrees of condensation of the skin tannins. The dark color near the surface represents the maximum degree of condensation (Ribereau-Gayon et al., 2000).

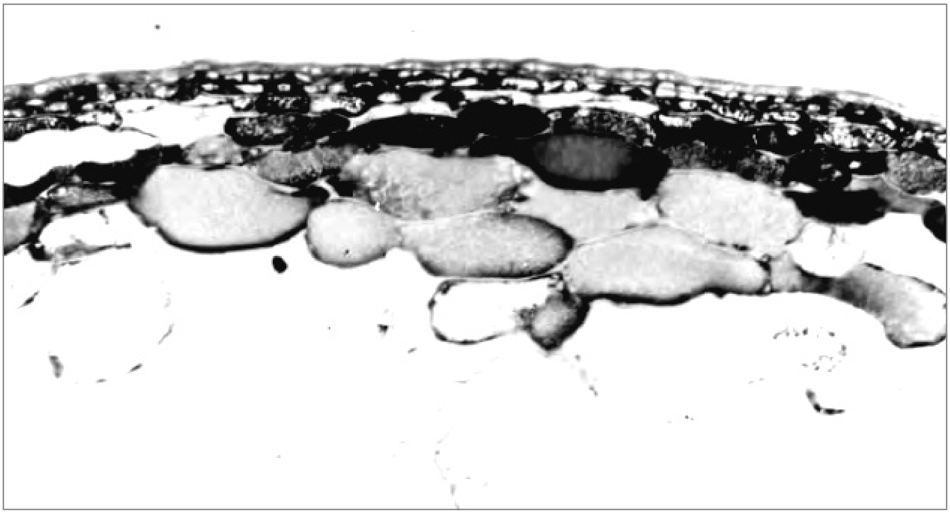

Figure 5. Cross-section of grape skin (Chardonnay variety). Observation under an optical microscope after staining with toluidine blue (x 207). Condensation of tannins at different levels in the skin. The darker-colored cells located near the surface of the skin correspond to the maximum condensation of tannins (Ribereau-Gayon et al., 2000a)

Phenolic maturity is a broader term that refers not only to the concentration of phenolic compounds but also to their structure and their ability to be extracted during alcoholic fermentation. Nowadays, there are sophisticated analytical methods to determine the phenolic maturity of grapes by calculating the content of potential anthocyanins and those that can be extracted into the future wine, as well as the proportion of the tannins of the seeds in the total phenolic compounds. Based on these data, it is possible to determine the phenolic maturity of the grapes and, consequently, the optimal harvest time.

What does modern oenology recommend, depending on the degree of grapes maturity?

In the case of unripe grapes

When the grapes are not fully ripe, the winemakers face a great temptation, because with a short maceration, the wine will not have the necessary color intensity, and with a long maceration there is a risk of increased extraction of astringent, herbaceous and bitter tannins. In such a situation, the oenologist has only two options: Harvest grapes with the correct sugar concentration (future alcohol) and optimal pH and adjust the technological processing procedure applied in the conditions of unripe grapes, or wait for the grapes to reach full ripeness and perform the harvest when the grapes are in the state of full ripeness, and then apply technological procedures to lower the alcohol content and pH.

Nowadays, consumers of red wines appreciate them when they are intensely red in color, rich in extract and balanced in taste. Therefore, a balance is sought between alcoholic sweetness (alcohol imparts sweetness at certain concentrations), sharpness and acidity. Excessive extraction of phenolic compounds during maceration affects the quality of red wines, making them tart and excessively astringent.

What does nature offer us depending on the ripeness of the grapes?

If we have unripe grapes and carry out a short maceration, we obtain a wine with a weak color that has no aging potential. On the other hand, with ripe grapes, a short maceration gives wines with appropriate color, structure, and fruitiness without bitterness, astringency and herbal notes. We obtain wines with intense and stable color, good structure and aging potential only from ripe grapes with a long maceration (Figure 6).

If the grapes are not yet fully ripe, the following operations and procedures may be helpful (Figure 7): Shortening the maceration period while promoting (accelerating) the extraction of anthocyanins; separation of seeds during the delestage process; applying microoxygenation of the wine or wine aging in oak barrels; enriching the wine with polysaccharides.

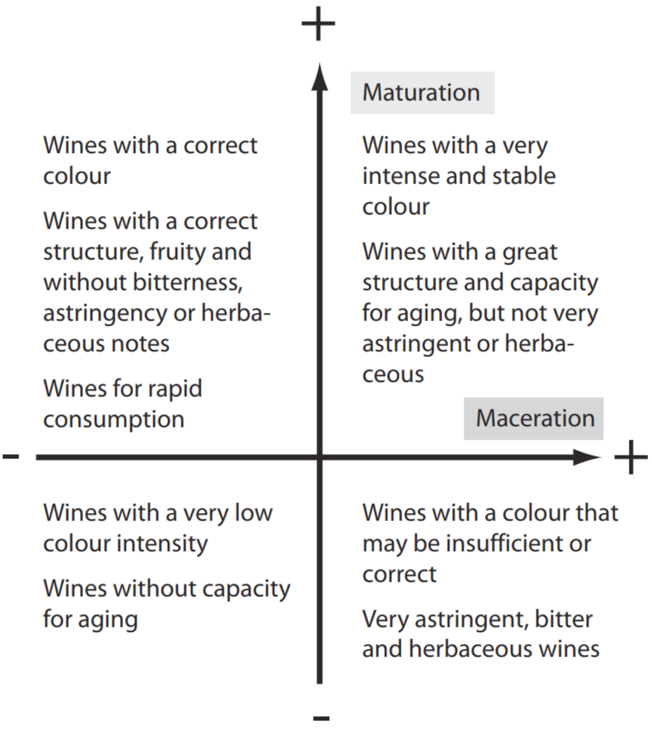

Figure 6. Relationships between maturation and maceration (Zamora, 2007)

When the wine remains in contact with the lees produced by the wine yeast after the end of alcoholic fermentation, it is enriched with polysaccharides. This technological process has a number of advantages, which are reflected in the following: the autolysis of the yeast cells increases the feeling of fullness of the wine in the mouth (mouthfeel); protection of the wine from oxidation; reduction of astringency; stabilization of the wine color; reduction of the influence of the wood on the wine; the emergence of new flavor components in the wine; increase in the persistence of the wine aroma. However, it should be remembered that the aging of wine on yeast sediment is associated with the following possible risks: risk of *Brettanomyces* proliferation; risk of reductive odor; slower development (maturation) of the wine. Moreover, by using selected wine yeast strains that release more polysaccharides, it is possible to obtain a wine with 30% more of these ingredients. In this case, these are mannoproteins, polymers of mannose (basic bond β (1-6)) with branching of other polysaccharides and peptide content below 30% (Ribereau-Gayon et al., 2000b). In the case of polysaccharides, there is the possibility of treating the wine with the so-called inactive yeast, which releases them into the wine.

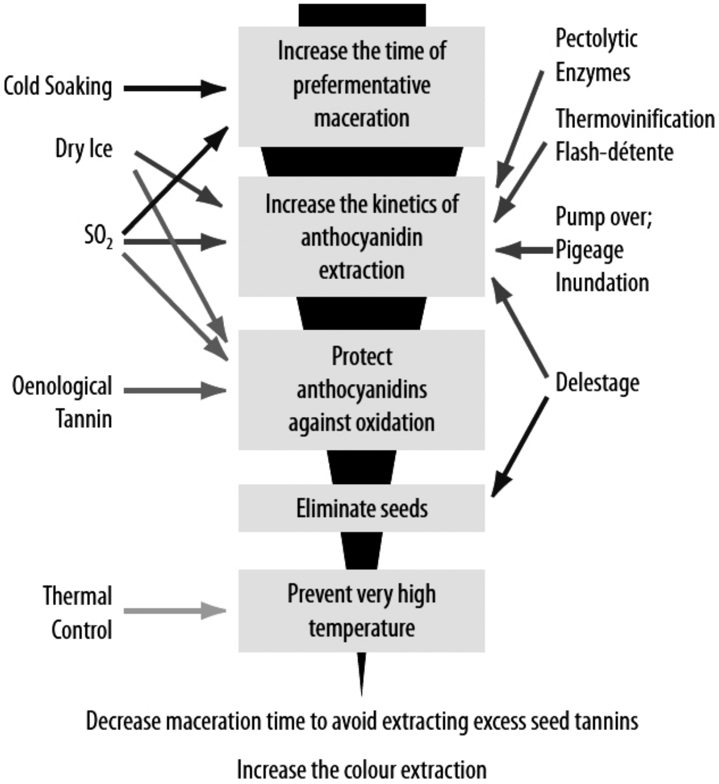

Figure 7. Possible winemaking strategies when the grapes are not sufficiently ripe (Zamora, 2007)

When the grapes are overripe

When the grapes are fully ripe, certain procedures and techniques are used to lower the alcohol content and pH. The options to be considered to correct the high alcohol content and high pH of wine are: selecting grape varieties and clones with late grape ripening time or planting vineyards at higher altitudes; adapting cultivation techniques to new conditions; selecting wine yeasts that produce less alcohol during alcoholic fermentation; use of glucose oxidase and peroxidase enzymes in the must; lowering the sugar concentration in the must by the process of reverse osmosis; partial dealcoholization of the wine – “spinning cone column”; lowering the pH of the wine by a cation exchanger or electrodialysis (Zamora, 2007).

When talking about climate changes, in addition to the problems already mentioned, which are reflected in a faster ripening of grapes, higher alcohol content of the future wine, lower acidity and an increase in pH, in our conditions, the following changes were also noticed: an increase in the protein content in grapes and must, intermittent disturbance of the mechanical composition of grapes, the appearance of sunburn in grapes of white varieties in the case of defoliation, and disturbances in photosynthesis in grapes of some varieties under conditions of prolonged drought.

It is known that at high temperatures, protein synthesis in the berry is more intense, resulting in a decrease in free amino acid content in favor of higher protein content. While in earlier years the use of bentonite at levels of 70 g/hl to 120 g/hl successfully removed all proteins from the must, today the bentonite requirement is 150 g/hl to 300 g/hl, depending on the variety, and in some cases significantly higher. High levels of bentonite also remove some of the free amino acids necessary to feed the yeast during alcoholic fermentation. For the above reasons, alcoholic fermentation often takes a very long time, which negatively affects the quality of the future wine, and it is not uncommon for fermentation to stop completely. For this reason, in recent years the application of nutrients for yeasts before and during alcoholic fermentation has become a common technological procedure.

At high temperatures, for objective or subjective reasons, there is often an over-ripening of the grapes, even a slight drying of the berries, which disturbs the normal mechanical composition of the grapes. In such cases, a decrease in the must content of the berries and a relative increase in the content of solid parts rich in phenolic compounds have been observed. For the above reasons, the maceration itself, which normally takes place before, during and after alcoholic fermentation, should be shortened so that the wines produced do not taste too aggressive due to the increased content of tannins and bitter substances.

It is known that under normal conditions, exposing grapes to direct sunlight by (partially defoliating) partially removing the leaves around the grapes results in a reduction of the grassy and herbaceous character of the aroma, while the fruitiness of the aroma remains unchanged or is somewhat more accentuated. It is precisely the increased content of monoterpenes produced in white Sauvignon under the influence of sunlight that contributes to masking the vegetative aroma produced by methoxypyrazines. From this point of view, 2-methoxy-3-isobutyl-methoxypyrazine (bell pepper aroma) in grapes decreases with increasing temperature. The content of rotundone in grapes, which is responsible for the peppery aroma of Shiraz, decreases with increasing temperature.

However, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), the compound responsible for the petroleum note of Rhine Riesling, increases with temperature as the grapes ripen. The content of linalool in grapes decreases at high temperatures, while this is not the case for geraniol. Massoia lactone (5,6-dihydro-6-pentyl-2(2H)-pyranone), the characteristic aroma of fig and coconut, is found in overripe fruit. For example, increased levels of massoia lactone are found in Bordeaux wines from Pomerol, which are made primarily from Merlot in warm years. An increase in C13-norisoprenoids in grapes due to drought is also noted. In dry years, Bordeaux Sauvignon Blanc contains more flavan-

3-ol and less glutathione. Glutathione is an antioxidant and increases the aging potential of white wines. High UV radiation stimulates the synthesis of anthocyanins, flavonols and tannins in black grapes, but can induce unpleasant aromas such as ortho-aminoacetophenone and the previously mentioned 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in white grapes (Van Leeuwen & Darriet 2016) (Figure 8).

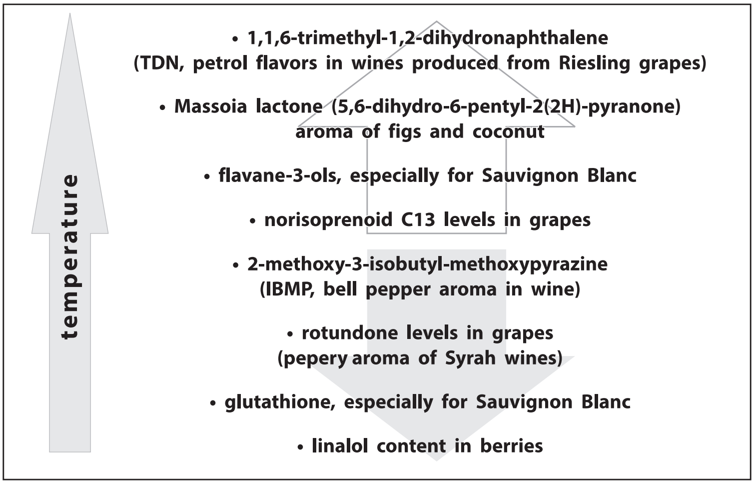

Figure 8. Influence of global warming on some aromatic compounds of white and red grapes and wines

CONCLUSION

About ten years ago, numerous papers were published dealing with the effects of global warming on the chemical composition and sensory properties of grapes and wine, as well as the search for methods to remedy the negative effects on wine quality. The increase in temperature as a result of the above changes is directly reflected in the earlier flowering of the vines, resulting in an earlier harvest. In the last 50 years, the harvest has moved forward by 20 to 30 days worldwide. In Serbia, harvesting is increasingly taking place in the month of August, when not all parts of the grape have reached the required maturity. As temperatures rise above normal, high concentrations of sugars accumulate in grapes, often causing acidity to drop significantly with an undesirable increase in pH. Phenolic maturity is a broader term that refers not only to the concentration of phenolic compounds, but also to their structure and their ability to be extracted during alcoholic fermentation. When the grapes are not fully ripe, the winemakers face a great temptation, because with a short maceration the wine will not have the necessary color intensity, and with a long maceration there is a risk of increased extraction of astringent, herbaceous and bitter tannins. If the grapes are not yet fully ripe, the following operations and procedures may be helpful: Shortening the maceration period while promoting (accelerating) the extraction of anthocyanins; separation of seeds during the delestage process; applying microoxygenation of the wine or wine aging in oak barrels; enriching the wine with polysaccharides. When talking about climate changes, in addition to the problems already mentioned, in our conditions, the following changes were also noticed: an increase in the protein content in grapes and must, intermittent disturbance of the mechanical composition of grapes, the appearance of sunburn in grapes of white varieties in the case of defoliation and disturbances in photosynthesis in grapes of some varieties under conditions of prolonged drought.

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ОРИГИНАЛНИ ЧЛАНАК

УТИЦАЈ ГЛОБАЛНОГ ЗАГРЕВАЊА НА САВРЕМЕНУ ЕНОЛОШКУ ПРАКСУ

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РЕЗИМЕ: Објављени су бројни радови који се баве утицајем глобалног загревања на хемијски састав и сензорне карактеристике грожђа и вина. Високе вредности температуре скраћују време потребно за бербу грожђа. Сви делови бобица нису могли да достигну пуну зрелост, а концентрација шећера у грожђу је у порасту. Киселост грожђа се често смањује заједно са нежељеним повећањем pH. Све ово утиче на бербу грожђа пре него што бобица достигне пуну технолошку зрелост, што се пре свега односи на покожицу и семенке. Недовољна зрелост грожђа утиче на хемијски састав и квалитет вина. Вино нема потребан интензитет боје. У случају дуге мацерације постоји ризик од повећања екстракције горких танина. У овој ситуацији, енолози морају да користе технолошки процес који се примењује у условима непотпуно зрелог грожђа или морају да сачекају пуну зрелост грожђа и затим примењују технолошке поступке за смањење садржаја алкохола и pH. Дакле, променљивост климе из године у годину утиче на квалитет грожђа, а самим тим и на квалитет вина.

КЉУЧНЕ РЕЧИ: глобално загревање, фенолна зрелост, вино

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ORIGINAL ARTICLE

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POTENTIALLY TOXIC ELEMENTS IN GRAPEVINE VARIETIES AND SOIL IN SERBIA

ABSTRACT: The accumulation of potentially toxic elements (PTEs) in agricultural soils and their subsequent uptake by plants, posing risks to agricultural products and consumers is of particular concern for investigation. Vineyard soils are especially vulnerable to pollution from various sources, including agrochemicals, fertilizers and surrounding polluting activities. This research is based on the BCR sequential extraction method to assess PTE speciation in vineyard soil samples. Additionally, it investigates the bioaccumulation of PTEs in grapevines (*Cabernet sauvignon* and *Sauvignon blanc*) and evaluates potential health risks for consumers of grapevine products based on each investigated PTE. The findings reveal that certain PTEs, Cd, Mn, Ba, Co, Pb and Cu exhibit significant mobility and bioavailability in vineyard soils, emphasizing the importance of continuous monitoring for food safety. The bioaccumulation potential, based on calculated biological accumulation concentration (BAC), exists for some elements, especially for Cu and Zn from easily available soil phase. The human health risk assessment indicates that the consumption of grapevine products poses minimal risk (HQ < 1) to consumers. Anyhow, among the investigated PTEs, Ba and Cu have the highest HQs. This research contributes to a better understanding of PTE dynamics in vineyard environments, emphasizing the need for ongoing monitoring and control measures to ensure agricultural product safety and protect consumers' health.

KEYWORDS: potentially toxic elements, soil, BCR sequential extraction, soil-grapevine bioaccumulation, risk assessment

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INTRODUCTION

Environmental pollution remains one of the most pressing challenges that humans and other living organisms are facing since it is disrupting normal environmental processes. Urbanization, industrialization and commercialization of rural areas have significantly impacted quality of the environment. The pollution of air, water, soil, and food has emerged as a global problem with far-reaching implications for human health (Muralikrishna and Manickam, 2017).

It is reported that among various pollution sources, agricultural activities have become a significant contributor to environmental degradation (WHO, 2018). The increasing utilization of agrochemicals globally has raised concerns about the accumulation of potentially toxic elements (PTEs) in agricultural soil and its subsequent uptake by plants. Exposure to the PTEs has prompted investigations into their potentially adverse health effects on humans (Pagano et al., 2015). Pollution from surrounding industrial activities, traffic, and improper waste disposal in rural areas can further exacerbate soil and product quality in agricultural areas. Escalating the presence of PTEs in the agricultural environment poses risks not only to agricultural products but also to the consumers (Milićević and Relić, 2021).

Vineyards, being a crucial part of agricultural landscapes, are particularly vulnerable to soil pollution caused by agrochemicals, fertilizers, pesticides and other industrial activities (Kabata-Pendias and Mukherjee, 2007). Monitoring of PTE concentrations in agricultural soil is an essential precaution for food safety, and studying the mobility and bioavailability of elements in the soil-plant-air system aids in understanding element uptake and translocation to plants, thus enhancing regulatory control of fruit and vegetable production (Milićević, 2018). Higher PTE concentrations in soils can pose health risks as they enter the food chain through plant uptake (Islam et al., 2015). Long-term consumption of food contaminated with PTEs can lead to severe health problems. Thus, comprehensive studies on the adverse effects of PTEs in vineyard environments and grapevine products are crucial.

In this study, we used the BCR sequential extraction (BCR SE), a widely used procedure for assessing the PTE speciation in vineyard soil samples; bioaccumulation of PTE in grapevine (*Cabernet sauvignon* and *Sauvignon blank*) was assessed by using biological accumulation concentration (BAC). Finally, in order to assess which of the PTEs has the highest influence on human health, this paper assessed potential health risks for each PTE, hazard quotients – HQs, for consumers of the grapevine.

MATERIALS AND METHODS

Sampling

The soil samples analyzed in this study were collected in the *Oplenac* grapevine growing region (44°13′36.3″ N 20°39′12.4″E), in the vicinity of *Topola* town.

Soil samples were collected from four locations (labelled 1, 2, 3, and 4; Figure 1) from two depths (0–30 cm and 30–60 cm). Soil samples were collected by the sampling probe. The studied parcels were in the system of no-tilling grapevine production and they were not located on sloping terrain. Sampling site 1 is located next to the winery and the main highway road in this region. Sites 2 and 3 are also located close to the main road. Site 4 is located behind a metal foundry, shielded from the main road (Figure 1). In this study, the composite soil samples from each site were studied. In total, 45 soil samples were collected from the vineyards during grapevine season. Two grapevine varieties (*Cabernet sauvignon* and *Sauvignon blanc*) planted at these parcels (in total 26 grapevine samples) were collected during harvest.

Figure 1. The sampling sites of soils and grapevines (44°13′36.3″ N 20°39′12.4″E)

Sample preparation and chemical analysis

In the laboratory, each soil sample was air-dried, sieved through a 2 mm stainless sieve and ground to a fine powder. The hygroscopic moisture, the acidity (pH) of soil and soil organic matter (SOM) are in detail described in the previous study (Milićević et al., 2018a). BCR sequential extraction consists of four steps. *First step:* 1g of each soil sample was extracted with 40 mL of 0.11 M CH_3COOH. The extraction was performed on a rotary shaker for 16 hours. *Second step:* To the rinsed residues, 40 mL of 0.5 M acidic aqueous $NH_2OH \times HCl$ solution was added to each cuvette, which was manually shaken with periodic openings. The extraction on a rotary shaker was performed for 16 hours. *Third step*: After rinsing, the residues were transferred from the cuvettes to glasses with 10 mL of 30% H_2O_2 and boiled for an hour in a water bath. Then, an additional 10 mL of 30% H_2O_2 was added, and boiling was continued for one more hour. Afterwards, the samples were transferred to the cuvettes using 1 M CH_3COONH_4 with a pH value of 2, and the extraction in the third BCR extraction step was performed for 16 hours. *Fourth step:* The residues were transferred from the vials to glasses and digested by aqua regia. Afterwards, the samples were filtered and neutralized with distilled water. The pseudo-total PTE concentrations were determined by microwave digestion of samples with aqua regia (details of this procedure were given in Milićević et al., 2017, 2018a, 2018b; Milićević, 2018).

The fresh grapevine samples were blended. The grapevine samples (1 g of fresh grape berries per each sample) of *Cabernet sauvignon* and *Sauvignon banc* were digested for 45 minutes in a microwave digester (ETHOS 1, Advanced Microwave Digestion System, Milestone, Italy) (US EPA 3050b, Method). The concentrations of Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, S, Sr, V and Zn in soil extracts (four phases of BCR SE), pseudo-total soil solution and grapevine berries digestion solutions were analysed using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

Data processing and statistical analysis

Our focus was to determine the speciation of PTEs in soil and grapevine, to use them in calculating the bioaccumulation from soil to grapevine and to assess human health risks. Statistical analysis was performed using SPSS software version 21. Kolmogorov-Smirnov test was used for testing normality of data ($p < 0.05$). Descriptive statistics were calculated and box and whisker plots were used for visualization.

For PTEs' bioaccumulation in grapevine, the biological accumulation concentration (BAC) was calculated for BCR sum values (Radulescu et al., 2013; Bravo et al., 2017) but also for the extracted concentrations in the 1st exchangeable phase of BCR SE which indicated the accumulation of bioavailable elements to plants. The biological accumulation concentration (BAC) was calculated using the following equations:

$$BAC_{\text{BCR sum}} = C_{\text{grapevine}} / C_{\text{BCR sum}}$$

$$BAC_{\text{1st BCR phase}} = C_{\text{grapevine}} / C_{\text{1st BCR phase}}$$

In this study, the risk of each PTE was observed for the first time, recognized as potentially toxic (US EPA 2007) for grapevine consumers (children, females and males) in this vineyard area. To find out which of the PTEs has the highest influence on the increase of the risk for human health, the HQs for each element were calculated for both investigated grapevine varieties. The equations for human health risk assessment are available at The Risk Assessment Information System, RAIS (RAIS, 2013).

RESULTS AND DISCUSSION

Potentially toxic elements in vineyard soil and their speciation

Table 1 presents descriptive statistics (Median, Standard Deviation, Minimum and Maximum) of PTEs extracted from soil by four phases of BCR SE whereas median values of BCR sum and median values of pseudo-total digestion done in microwave digester were compared. The pseudo-total and BCR sum concentrations of PTE in soil were measured in concentrations lower than the maximum allowed concentrations (MAC, National Gazette of the Republic of Serbia). Only pseudo total Cr and Ni were determined in higher concentrations than MAC and Ni BCR sum was slightly higher than MAC (Table 1). These elements usually come from the soil's parent rock. The Balkan Peninsula's soil (Salminen et al., 2005), per the Geochemical Atlas of Europe, has naturally higher Cr and Ni levels. In the vineyard's topsoil from this area, Cr and Ni did not correlate with Fe concentration, unlike the subsoil where they did (Milićević et al., 2018a). Chromium mobility in the soil depends on Fe content and pH value in this vineyard where the soil has neutral pH and low organic matter (Milićević et al., 2018a). High Cr and Ni concentrations in both soil layers likely result from both natural and human factors, which are proved by significant correlations between the concentrations of these elements in the vineyard soil (Milićević, 2018). BCR sequential extraction has proven to be the suitable method for assessing the potential mobility of PTEs, with four main soil fractions identification (water acid-soluble and carbonate fraction; reducible fraction bound to Fe and Mn oxides and hydroxides; oxidizable fraction bound to organic matter and sulphides; and residual fraction bound to crystalline substrates in the soil).

Observing the results of the first phase of BCR SE (0.11 M CH_3COOH), which represents water acid-soluble and carbonate fraction of soil, the highest concentrations among the investigated elements of Sr (47%), followed by Ca (55%), and Cd (31%) were extracted comparing to the BCR sum concentration. These results suggest the dissolution of Sr and Ca carbonates, along with the release of ion-exchangeable from the soil substrate. The presence of Cd (33%) in the most mobile fraction is noteworthy, indicating its potential mobility under mild acid conditions and the possibility of entering the food chain. Elevated Cd concentrations in the topsoil (0–30 cm) compared to the subsoil (30–60 cm) were obtained in previous research (Milićević et al., 2018b) that suggests Cd association with the use of phosphate fertilizers or other agrochemicals containing it. Cadmium, highly toxic and excessive in the topsoil, can originate from fertilizer use, impacting life and food quality (Kabata-Pendias and Mukherjee, 2007). Based on the results it can be noted that Cd is present in low concentrations (lower than MAC both pseudo total and BCR sum; Table 1) but due to its high potential bioavailability, its bioavailability to grapevines and bioaccessibility to humans should be more studied relying on the environmental and human health risk methods.

Table 1. The concentrations (mg/kg) of PTE extracted by SE (1st phase: water acid-soluble and carbonate fraction; 2nd phase: reducible fraction bound to Fe and Mn oxides and hydroxides; 3rd phase: oxidisable fraction bound to organic matter and sulphides; and 4th phase: residual fraction bound to crystalline substrates in the soil); sum of all phases of BCR SE, pseudo total concentrations, recovery of BCR SE.

	Ba	Be	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	P	Pb	S	Sr	V	Zn
1st Phase: 0.11M CH_3COOH (n=45)																	
Median	18.2	0.14	2905	0.04	0.48	0.08	0.42	1.97	281	66.1	2.03	2.42	0.02	10.41	7.25	0.013	0.51
SD	3.83	0.05	1525	0.01	0.23	0.34	0.95	2.48	63	20.8	0.51	8.05	0.03	4.69	16.3	0.008	0.92
Min	9.62	0.00	932	0.02	0.05	0.01	0.01	0.34	136	32.2	0.72	0.13	0.01	1.09	3.96	0.001	0.003
Max	23.0	0.19	7281	0.06	0.91	1.69	4.77	10.71	389	128	2.62	35.76	0.13	18.48	89.0	0.042	5.20
Median % of BCR SUM	14.6	7.25	55	31.2	2.84	0.15	1.26	0.01	7.39	8.72	2.84	1.08	0.08	7.38	47	0.043	1.22
2nd Phase: 0.5M $NH_2OH \times HCl$ (n=45)																	
Median	73.7	0.54	2630	0.05	8.76	3.01	7.02	1659	359	490	10.3	21	16.6	<DL	5.07	6.40	7.22
SD	12.7	0.09	1720	0.01	2.55	1.98	8.08	508	78	110	2.73	24	2.70	NA	3.40	1.18	2.98
Min	45.0	0.33	1382	0.02	6.03	1.35	2.97	1036	208	313	3.16	0.07	9.96	NA	4.18	4.30	3.02
Max	98.6	0.70	12055	0.08	18.5	12.97	37.2	3094	540	851	15.7	101	22.7	NA	26	9.11	15.82
Median % of BCR SUM	65	29	44	45	59	5.39	21.2	8.66	8.97	69	15.2	8.80	57	NA	35	21	17
3rd Phase: H_2O_2 + 1M CH_3COONH_4 (n=45)																	
Median	7.57	0.15	122	0.03	0.83	10.13	2.99	435	344	32.22	7.63	< DL	4.04	124	0.34	4.70	2.85
SD	1.91	0.04	64	0.01	0.14	5.98	1.74	137	65	11.11	2.63	NA	1.00	26	0.12	1.52	1.42
Min	4.22	0.09	67	0.001	0.62	5.68	1.46	210	242	13.70	3.50	NA	2.20	65	0.23	0.52	1.01
Max	12.47	0.22	437	0.07	1.30	37.30	10.70	862	524	53.69	16.88	NA	7.79	168	0.98	7.94	7.74
Median % of BCR SUM	6.37	8.35	2	24	5.10	19	8.35	2.30	9.0	4.31	12	NA	14	92	2.22	16	6.69
4th Phase: Aqua regia (n=45)																	
Median	15.43	1.07	52	NA	4.88	44	23.16	18560	2881	113	48.80	204	8.21	< DL	2.34	18.58	31.2
SD	4.30	0.16	63	NA	1.46	13	4.81	3357	679	22	15.01	47	1.28	NA	0.73	3.71	4.7
Min	5.70	0.63	0.37	NA	2.62	13	15.8	10687	1459	77	12.49	117	5.12	NA	0.76	11.15	19.5
Max	25.0	1.31	288	NA	8.26	76	35.8	22605	4299	183	71.12	323	10.66	NA	3.68	29.33	37.0
Median % of BCR SUM	14	57	0.95	NA	32	76	69	89	74	17	69	89	28	NA	12	63	74
Median BCR SUM	115	1.8	5603	0.1	15.6	58.1	35.0	20546	3876	685.5	67.8	246	29.1	134.4	15.0	30.1	43.7
Pseudo-total (PT) concentration																	
Median PT	243	1.9	6950	0.38	23.8	133	43.7	42206	8499	1056	105	337	31.4	104	41.7	63.9	82.3
MAC				3		100	100				50		100				
Recovery of BCR%	47	95	81	26	66	44	80	49	46	65	65	73	93	130	36	47	53

In the second phase of BCR SE the concentrations of Mn (69%), Ba (65%), Co (59%), Pb (57%), Cd (45%) and Ca (44%) and Sr (26%) were most efficiently extracted comparing to the BCR sum value (Table 1). The second phase (0.5 M $NH_2OH \times HCl$) simulates conditions for the reductive dissolution of Mn oxides and amorphous Fe oxides. The total Mn content is primarily from the parent substrate, as significant Mn oxides were destroyed in this phase. However, anthropogenic influences, such as use of Mn-based fungicides, should also be considered (Milićević et al., 2018a). In the literature, elevated concentrations of Mn, Ba, and Co were measured in soil samples, likely influenced by proximity to households and local roads, which can emit anthropogenic Co, particularly from the combustion of oil, coal, and agricultural machinery exhaust gases (Kabata-Pendias and Mukherjee, 2007).

In the third phase BCR SE the extracted concentrations of S (92%), and then Cr (19%) and Pb (14%) were higher than others compared to the BCR sum. Other extracted PTEs in this phase represent less than 10% of BCR sum concentrations. This result was expected due to the fact that this phase causes oxidative destruction of mainly organic matter and some extent of sulphides in soil.

In the fourth phase, after digestion residues with aqua regia, the highest content of Fe (89%), P (84%), Mo (81%), K, Al (78%), Mg, Cr (75%), Li, Zn (72%), Ni (70%), Cu (65%), V (63%), Be (57%) and B (51%) were extracted comparing to the sum of BCR extracts. These elements are bound to crystalline substrates in the soil, and their mobility, especially for toxic elements, requires more drastic conditions (very low pH) to dissolve and release them into the soil solution.

Recovery of BCR sum compared with aqua regia microwave digestion procedure is not efficient for all investigated elements. The moderate to high recovery (from 65% to 130%) was obtained for Be, Ca, Co, Cu, Mn, Ni, P, Pb, and S. For other elements, the recovery compared to pseudo-total concentration is lower. These results are not unusual due to the fact that BCR sequential extraction is performed in an open system where some losses in concentration are expected compared to pseudo-total concentration of soil in microwave digester (Relić et al., 2013, 2018). Anyhow, the BCR sum concentrations significantly correlated with concentrations of the same soil samples digested with aqua regia in a microwave digester ($r=0.971$; $p<0.01$ and regression coefficient $R^2=0.989$; Figure 2). For the speciation of PTEs mobility and bioavailability to plants is of the major importance to assess the element speciation. Pseudo-total element content by adding up all four phases of BCR SE is significantly lower than microwave-digested samples in the closed system, but anyhow the results are comparable (Figure 2).

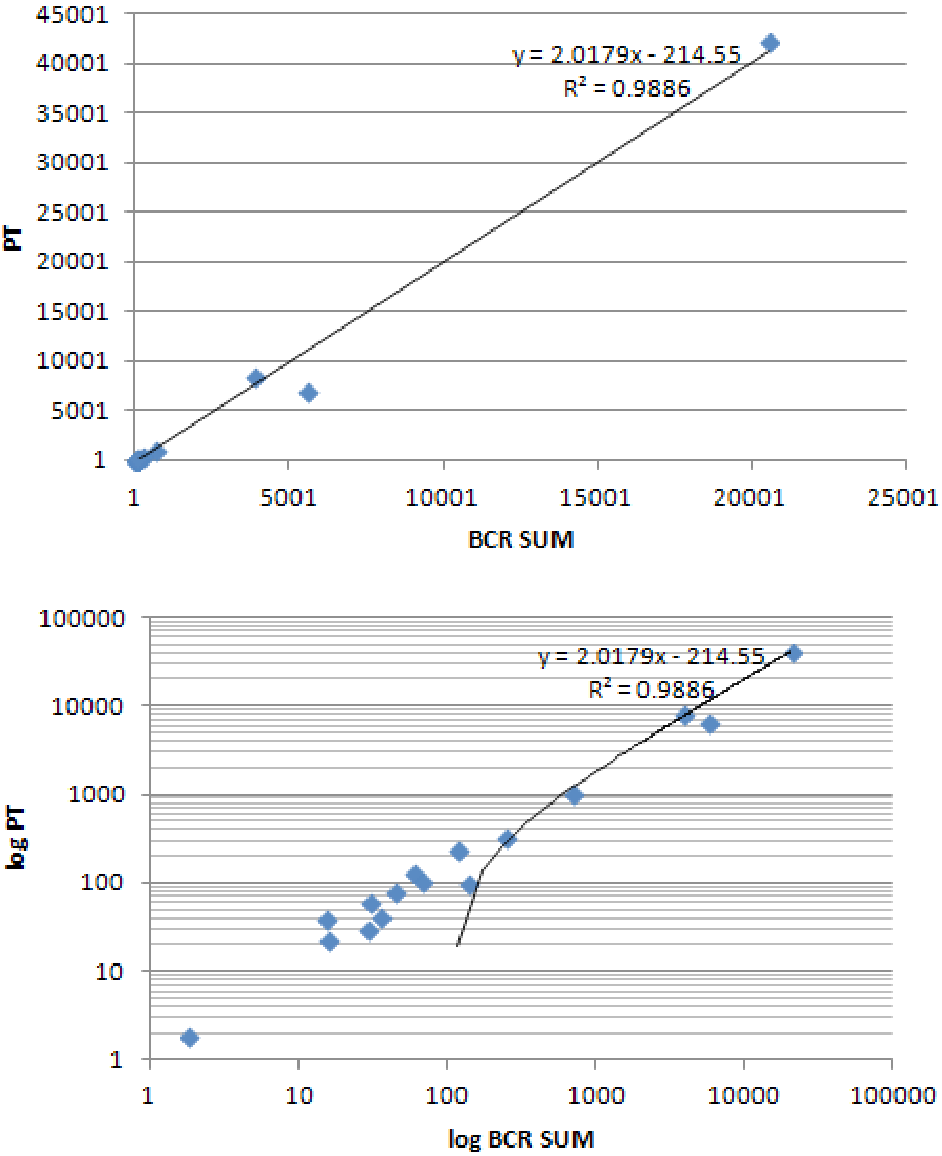

Figure 2. The regression analysis between the concentrations (mg/kg) obtained after microwave digestion with aqua regia (pseudo-total – PT) and BCR SUM concentrations (mg/kg)

Bioaccumulation of potentially toxic elements in the grapevine varieties from soil

The concentrations of PTE obtained in grapevine varieties (*Cabernet sauvignon* and *Sauvignon blanc*) are lower than the MAC (Milićević et al., 2018a). Anyhow, the investigation of the bioaccumulation of elements from soil to plant is of high importance due to production management and dosing chemicals. Observing the results, it can be noticed that there are no significant differences in the BAC values calculated for topsoil (0–30 cm) and subsoil (30–60 cm), as well as between the two investigated varieties (Figure 3). We assessed the bioaccumulation of PTEs from four investigated soil phases (BCR sum) to grapevine (*Cabernet sauvignon* and *Sauvignon blanc*) and also the bioaccumulation of the easily available PTE fraction from vineyard soil to the grapevine. The bioaccumulation based on the BCR sum concentrations showed that for all elements BACs are lower than 1, anyhow the values of BAC calculated for Zn and Cu indicated that there is higher ability for bioaccumulation of these elements in the grapevine (Figure 3a). It can be noticed that BAC from easily available 1st SE fraction implies that from bioavailable soil fraction Cu, Fe, Mg and Zn are easily available and bioavailable to plants (Figure 3b). Furthermore, there is a slightly increased BAC than one for Ca and S. Thus, the speciation of these elements by SE extraction could be helpful, to identify their mobility and bioavailability. From the soil bioavailable fraction, Zn and Cu are the most bioaccumulated to grapevine, while the bioaccumulation potential is significant for Fe, Mg, Ca and S. All these elements are necessary for the plant growth, but their bioaccumulation, bioavailability and human health risk some of them (especially Cu) can cause should be more monitor and controlled in vineyard ambient.

The human health risk assessment based on the PTE in grapevine

The hazard quotient HQ (the risk factor for assessment of the non-carcinogenic risk) for each PTE was calculated for children, female and male adults who consume the investigated grapevines. Even this grapevine is primarily used in wine production; anyhow the direct consumption of fresh berries could be of great importance for the fruit consumers. Based on the results (Figure 4) it can be noticed that there is no risk for consumers of these two varieties of grapevine. All the HQ values for investigated PTEs are lower than one.

Anyhow, it can be seen that the highest HQs among the investigated PTEs have Ba and Cu. These elements should be investigated and monitored in the vineyard ambient not only due to the production of the grapevine but also because of the potential influence on the increase of risk to human. Moreover, it can be noticed that two outlier HQs for Cu are significantly higher than others in *Cabernet sauvignon* from location 4 (Figure 1). Finally, this risk assessment represents the worst-case scenario for frequent and uncontrolled consumption of grapevine. Our results show that these varieties are safe for frequent consumption.

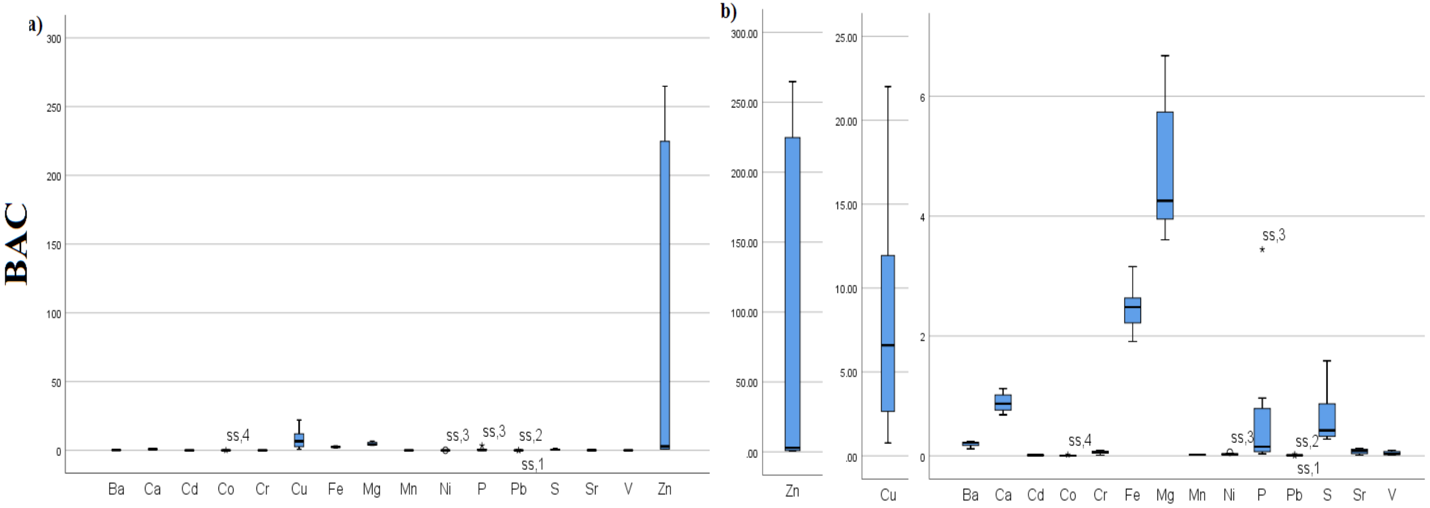

Figure 3. Biological accumulation concentration (BAC) calculated based on the PTE concentrations in grapevine and soil: a) all BCR SE phases as BCR sum, b) 1st phase BCR SE and varieties

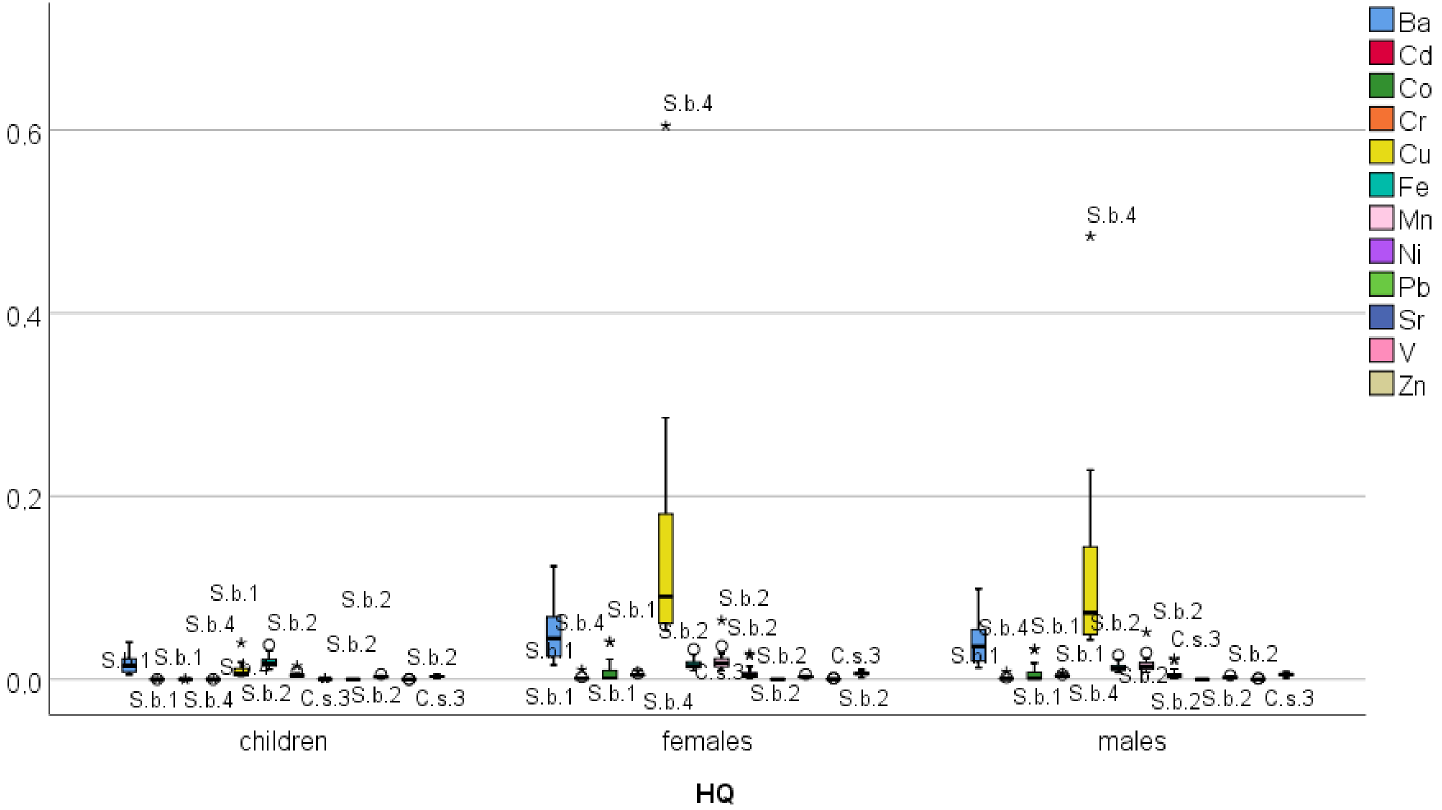

Figure 4. The hazard quotient (HQ) for PTEs (S.b. is *Sauvignon blanc* and C.s. is *Cabernet sauvignon*)

CONCLUSION

This study assessed PTE speciation in vineyard soil samples using the BCR sequential extraction method. Additionally, it investigated the bioaccumulation of PTEs in grapevines (*Cabernet sauvignon* and *Sauvignon blanc*) and evaluated potential health risks for consumers of grapevine based on each determined PTE.

The findings reveal that certain PTEs, such as Cd, Mn, Ba, Co, Pb, and Cu, exhibit higher potential for mobility and bioavailability in vineyard soils than other PTEs, indicating their potential to enter the food chain. These results emphasize the need for comprehensive monitoring of PTE concentrations in agricultural environments to ensure food safety. While the concentrations of PTEs in grapevine varieties were found to be within permissible limits, the bioaccumulation potential of some elements, notably Cu and Zn, warrants attention. Nevertheless, the human health risk assessment indicated that the consumption of these grapevine varieties does not pose a significant risk to consumers, with hazard quotient values well below 1 for each investigated element in the grapevine. Anyhow, the elevated HQ values for Ba and Cu among the examined PTEs suggest the need for increased monitoring and human health risk assessments based on these elements in various grapevine varieties. This is crucial for implementing control measures to guarantee the safety of agricultural products and safeguard human health.

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ПОТЕНЦИЈАЛНО ТОКСИЧНИ ЕЛЕМЕНТИ У СОРТАМА ВИНОВЕ ЛОЗЕ И ЗЕМЉИШТУ У СРБИЈИ

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РЕЗИМЕ: Акумулација потенцијално токсичних елемената (ПТЕ) у пољопривредним земљиштима и њихово усвајање од стране биљака представља ризик за пољопривредне производе и конзументе истих. Виногради су посебно изложени загађењу земљишта из различитих извора, укључујући агрохемијске препарате, ђубрива и околне активности које могу довести до повећања концентрација ПТЕ. У оквиру овог истраживања применили смо *BCR* секвенцијалну екстракцију како би проценили специјацију ПТЕ у узорцима земљишта из винограда. Такође, процењена је биоакумулација ПТЕ у виновој лози (Каберне совињон и Совињон блан) и процењен је потенцијални здравствени ризик за конзументе винове лозе на основу сваког испитаног ПТЕ. Резултати откривају да одређени ПТЕ, као што су Cd, Mn, Ba, Co, Pb и Cu, показују значајну мобилност и биодоступност у земљиштима из винограда, што указује на важност континуираног мониторинга ових елемената у виноградима. Потенцијал за биоакумулацију, заснован на израчунатим биолошким акумулационим концентрацијама (*BAC*), је повећан за неке елементе, посебно за Cu и Zn из лако доступне фракције земљишта. Процена ризика по здравље људи указује да конзумирање грожђа има занемарљив ризик по здравље конзумената ($HQ < 1$). Међутим, највише *HQ* вредности међу испитивним ПТЕ имају Ba и Zn. Ово истраживање доприноси бољем разумевању биодоступности ПТЕ у амбијенту винограда, наглашавајући потребу за континуираним праћењем и мерама контроле ради осигурања безбедности пољопривредних производа и заштите здравља конзумената.

КЉУЧНЕ РЕЧИ: потенцијално токсични елементи, BCR секвенцијална екстракција, земљиште-винова лоза биоакумулација, процена ризика за конзументе

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ORIGINAL ARTICLE

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PRODUCTION AND TECHNOLOGICAL CHARACTERISTICS OF THE MINOR GRAPEVINE VARIETY *SREMSKA ZELENIKA*

ABSTRACT: Minor grapevine varieties represent enormous value in terms of cultural heritage, diversification of products with higher economic value and adaptation to environmental conditions. This paper presents the results of research on certain production and technological characteristics (mechanical composition of clusters and berries, as well as chemical composition of grape must) of a local, i.e. minor white wine variety *Sremska Zelenika* examined in the agro-ecological conditions of the Fruška Gora wine-growing sub-region (Srem wine-growing region) in the locality of Sremski Karlovci. In the two-year research (2020 and 2021), without any yield reduction, it was determined that the average mass of the cluster was 266.5 g. The average sugar content in the grape must of the tested variety was 19.8%, the average content of total acids was 6.75 g/l and the average pH of the grape must was 3.27. Comparing the results of previous researches conducted in the same locality, it is concluded that in this research the minor variety of *Sremska Zelenika* has some better production and technological characteristics.

KEYWORDS: *Sremska Zelenika*, Fruška Gora wine-growing sub-region, production and technological characteristics

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is, economically, the most important fruit species in the modern world (Vivier et al., 2002). This claim also applies to Serbia, as the first reported occurrence of *Vitis vinifera* in the Balkans in the form of wild grapes dates back to the Neolithic period (Burić, 1972).

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The turbulent history of the Balkans and Serbia and change of different cultures resulted in rises and falls in the development of viticulture. Thus, rare traditional varieties used in the past have been forgotten after modern varieties were discovered. They are particularly endangered and their existence in vineyards have been gradually decreasing. Cindrić et al. (1997) note that commercially inferior varieties were gradually pushed out of production with a high probability of being lost forever. In the past, the now minor varieties used to be the leading varieties in the Fruška Gora wine-growing sub-region (Lazić, 1982). Apart from other local (indigenous and regional) old varieties, a similar situation, i.e. gradual clearing of vineyards also happened with the *Sremska Zelenika* minor variety.

However, the data show that it is necessary to preserve genetic diversity and maintain the gene pool at least at the current level. Traditional varieties are well adapted to local conditions and therefore valuable for breeding modern varieties, but they are also interesting from a historical perspective, where they have their place at open-air exhibitions presenting varietal diversity and the history of viticulture (Baránková et al., 2020).

Minor grapevine varieties have an enormous value in terms of cultural heritage, diversification of products with higher economic value and adaptation to environmental conditions. Over the last 20 years, the reputation of minor grapevine varieties has considerably increased (Maul et al., 2019). Countries like Italy and Spain have long been using the potential of their indigenous varieties and, with modern growing cultivation, constant yield control and good oenological practices, they produce wines that are known throughout the world (Maletić et al., 2012). In the last period, in Serbia, wines have been increasingly produced from local grapes, i.e. minor varieties of the vinegrape, and some research has being done on certain varieties, such as *Grašac*, *Prokupac*, *Bagrina*, *Smederevka*, *Tamjanika* (a group of varieties) and others (Korać et al., 2007; Rakonjac et al., 2010; Ivanišević et al., 2012; Jakšić et al., 2023)

Although mentioned in the literature, the local, or minor, variety of *Sremska Zelenika* is not present in large areas of Serbia or in the region (Galbács et al., 2009; Lázár and Bisztray, 2011; OIV, 2017). In terms of surface area, this variety holds the 107th place when it comes to distribution in commercial vine plantations and the only vineyards intended for commercial grape production are in the wine-growing regions of Subotica and Srem. The vineyards with this variety are located at an altitude in the interval of more than 100 m to 200 m, on the eastern and somewhat less on the southern exposures of the terrain. They are located on terrain slopes with inclination of more than 1 º to 5 º, and the average area of commercial vineyards with this variety is only 0.07 ha (Jakšić et al., 2019).

The *Sremska Zelenika* variety originates from the area of the Pannonian Plain (Cindrić et al., 2000), which is confirmed by the geographical designation "Sremska" in the name of the variety. In the international database Vitis International Variety Catalog (VIVC) (https://www.vivc.de/) it is stated that the origin of this variety is from the Balkans. According to literature data, the synonym of this variety in Serbia is *Zelenika*, while in Hungary the synonym

Szerémi Zöld is used (Cindrić et al., 2000). In the VIVC database (https://www.vivc.de/), in addition to the name *Sremska Zelenika*, 17 synonyms are listed, namely: *Gruene Spitzler; Gruener Schitzler; Gruenspitzler Matarski Riesling; Serem yellow; Spaetgreen; Spaetgruen the whiteness of Srem; Sheremi Szeremi Zoeld; Szrem whiteness; Szrem Zlte; Teremi; Teremi Zoeld; greens; Zoeld Szeremi; Zoeldszeremi*, which may indicate there was widespread cultivation of this variety in the region in the past. As many as eight of the listed synonyms have the geographical term "Srem". Through genetic identification, it was determined that the parent pair of this variety is *Purcsin* x *Francuse* (https://www.vivc.de/). According to some authors, the *Sremska Zelenika* variety was grown in Yugoslavia, i.e. Serbia, Hungary, and Romania (Cindrić et al., 2000; 1994), and there is information that it was also grown in the Zakarpattia region in Ukraine, where grapes, due to high acid content, were used for production of sparkling wines. However, in all these countries of the region, as well as in Serbia, it has gradually disappeared, so that today it is classified as a minor variety, present on in less than 2% of the total area of vine plantings (OIV, 2017).

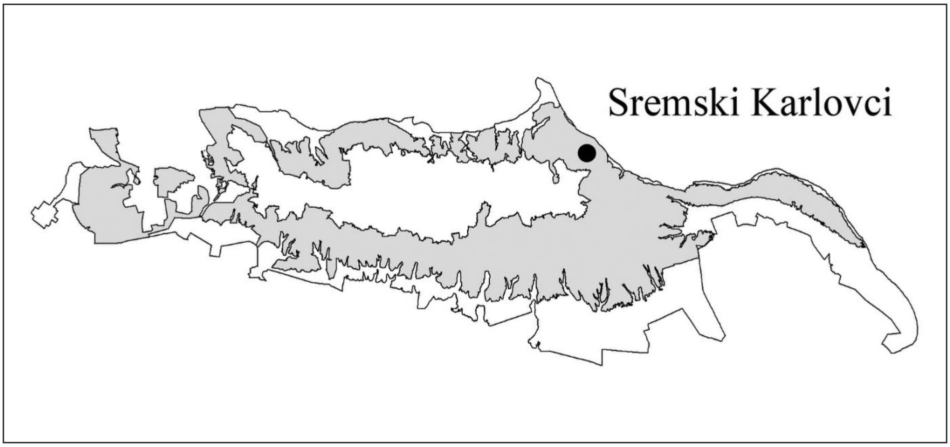
The variety *Sremska Zelenika* is characterized by great vigor. It has characteristic deeply incised and serrated leaves. The bunch (cluster) is medium-sized and compact. The berries are medium-sized, round, green in color, with a very sour taste. It is a late-maturing and high-yielding variety (Cindrić et al., 1994; 2000). Grown in unfavorable agroecological conditions, vine plants of this variety can freeze a lot (Lázár and Bisztray, 2011), however, the variety is high-yielding, which may favor grape quality over quantity. In unfavorable years, due to the compaction of the berries, the grapes may be susceptible to rotting (Lázár and Bisztray, 2011), which could be one of the reasons for gradual clearing of vineyards with this variety. When this genetically high-yielding variety is grown in unfavorable localities, its grapes can have a lower sugar content and a very high acid content; however, when well ripened, it produces a very solid wine with a mild aroma (Németh, 1967; Lázár and Bisztray, 2011; Cindrić et al., 2000). However, one should keep in mind that earlier research was carried out in less favorable, colder conditions and that the climate in Serbian wine-growing areas has shifted towards higher temperatures. Such changes mostly go in favor of growing grapevine varieties with a later ripening time (Jakšić et al., 2023), such as the minor variety *Sremska Zelenika*. Due to a higher content of acids in the grape must and a fresh character of the wine, this variety may deserve more attention in future scientific research and production in conditions of climate change and increased temperatures.

Until now, in Serbia, no work has been done on the clonal selection of the *Sremska Zelenika* variety, or on detailed systematic testing in terms of the application of various measures to reduce the yield and increase the quality of grapes and wine. For that reason, the goal of this work was to examine certain production and technological characteristics of the *Sremska Zelenika* variety, to explore a possibility for spreading this variety in the conditions of the Fruška Gora wine-growing sub-region and other areas with favorable agro-ecological conditions in the future.

MATERIALS AND METHODS

Object of the research

The research was carried out on estate demonstrational vineyard of the Department for fruit growing, viticulture, horticulture and landscape architecture of the Faculty of Agriculture of the University of Novi Sad, located in the Srem wine-growing region, the Fruška Gora sub-region (Map 1).



Map 1. Srem wine-growing region, Fruška Gora sub-region, and location of vineyards

The two-year research (2021 and 2022) of the production and technological characteristics of the *Sremska Zelenika* variety was carried out in a vineyard, i.e. a collection plantation set up in 2008. The planting distance between the rows in the vineyard was 2.8 m, and between the plants in the rows was 1 m. The research on selected production and technological characteristics of the exanimated grapevine variety was carried out on a sample of ten grapevine plants. The plants were grafted onto *Berlandieri* x *Riparia* Kober 5BB. The training system was a modified Single Guyot, i.e. the Karlovci training system, where long canes were left at the top of a tall trunk, bent in a semicircle and tied to the lowest wire (Cindrić et al., 2000) (Picture 1). The time of phenological maturity of the tested variety, i.e. the harvest date in the first year of research (2021) was October 5th, while in the second year of research (2022) it was September 26th.

The predominant type of soil on which the vineyard is located is eutric cambisol, and there are also other types of soil, to a lesser extent. The terrain has a very slight slope from the south to the north, in which direction the rows were set up. The collection of grapevine varieties, where the research was done, is located at an altitude of 110 m (45°11’30.3” N; 19°55’58.1” E).

Picture 1. Sremska Zelenika variety

The agrotechnical and ampelotechnical measures applied in both years of the research were not different from the measures applied in the whole collection plantation. Therefore, the grapes were not reduced, but were allowed to express the full genetic potential of the variety.

METHODS

The researchers used the method of selecting representative grapevine plants for this variety and chose ten grape clusters. To ensure the same load, the same training method was carried out during dormancy, where 12 buds were left on the canes and two buds on the spurs.

When it comes to the production and technological characteristics of the exanimated variety, the mechanical composition of clusters and berries, as well as the chemical composition of the grape must were determined.

The mechanical analysis of the clusters and berries (chemical analysis) was done by using a modified Prostoserdov method (Prostoserdov, 1946). In laboratory conditions, the structural composition of the cluster was determined: length (cm), width (cm), and mass of the cluster (g), as well as the number and mass of all berries in the cluster (g). From the structural indicators of the berry, namely the mass of 100 berries (g), length (cm), and width of the berry (cm) were also determined. To investigate the mechanical composition of the grapes, ten representative clusters from all selected vines were used.

The grape must for determining the chemical composition was obtained by mixing and crushing 100 berries taken from the 10 representative clusters. The researchers determined the following parameters of the chemical composition: the content of sugar and the content of total acids in the grape must, as well as the pH of the grape must. The content of sugar in the grape must was determined by an electronic refractometer and expressed in %. The content of total acids was determined by using the method based on the color change of the indicator. This method is based on titration with NaOH solution in the presence of a phenolphthalein indicator. The acid content was expressed in g/l of tartaric acid. The pH of the grape must was determined using a pH meter. The method is based on the measurement of a potential difference between two electrodes immersed in the tested liquid. The result was expressed with an accuracy of 0.05 pH units.

Using the method of questionnaires, 4 growers of the *Sremska Zelenika* grapevine variety from different wine-growing regions were inquired on the average sugar content in 2021 and 2022.

RESULTS AND DISCUSSION

The important production and technological characteristics in the period 2021–2022 for the wine grapevine variety *Sremska Zelenika* are shown in Tables 1, 2 and 3, as well as in Graphs 1 and 2.

Mechanical composition of the cluster

The research determined that the mass of the cluster in the first year (2021) of the research was 289.2 g, and in the second year (2022) was 243.80 g, while the mass of the cluster of the exanimated variety for both years was 266.5 g (Table 1). The mass values of ten clusters from this research are slightly higher than the ones recorded in previous studies in the Fruška Gora wine-growing sub-region in the same location (Cindrić et al., 2000) in the period 1981–1998, when the average mass of the cluster not only of the *Sremska Zelenika* variety, but also of all the examined plants from the collection was 244 g. The determined average number of berries in a cluster of the *Sremska Zelenika* variety was 173.

The research has established that the cluster of larger size was recorded in the first year (2021), with the length of 16.33 cm, and the width of 10.33 cm. In the second year of the research (2022), the cluster was somewhat smaller in size being 15.50 cm long and 9.00 cm wide. The average length for both years was 15.92 cm, while the average width was 9.67 cm.

Table 1. Grape characteristics of the *Sremska Zelenika* variety

Year	Cluster mass (g)	Cluster length (cm)	Cluster width (cm)	Number of berries in a cluster
2021	289.20	16.33	10.33	165
2022	243.80	15.50	9.00	181
Average	266.50	15.92	9.67	173

Analyzing the results of the research, there were some differences between years observed in the following characteristics of the grape cluster: cluster mass (g), length and width of a cluster (cm), as well as the mass and number of all berries in a cluster (g). For all tested characteristics of mechanical composition of a cluster, higher values were recorded in the first year of the research (2021).

Results of the mechanical composition of the berry

The mass of 100 berries (g) in the examined variety *Sremska Zelenika* was different. Namely, this value of mechanical composition ranged from 179.10 g (first year of examination) to 108.30 g (second year of examination). The average value of the mass of 100 berries for both years of the *Sremska Zelenika* variety was 143.70 g (Table 2).

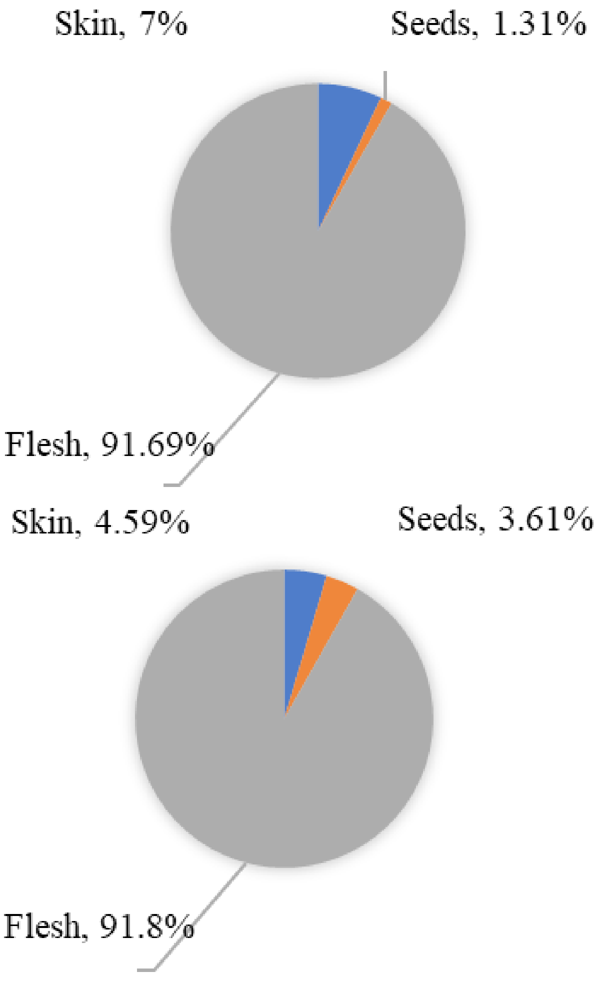
Table 2. Properties of the berry of the *Sremska Zelenika* variety

Year	Mass of 100 berries (g)	Berry length (mm)	Berry width (mm)
2021	179.10	13.11	13.38
2022	108.30	12.50	12.68
Average	143.7	12.81	13.03

In the first year (2021), the berries were larger, being 13.11 mm long and 13.38 mm wide, while the average length of the berries for both years was 12.81 mm and the width 13.03 mm.

The results of the structural indicators of the clusters and berries

The research determined that the percentage of flesh in 100 berries was approximately the same in both years. In the first year, the value was slightly higher (91.8%) than in the second year (91.69%). Skin-wise, a significantly lower percentage of the skin was recorded in the first year (4.59%) than in the second year (7%) (Graphs 1 and 2).



Graphs 1–2. Percentage of skin, flesh and seeds in a berry of the *Sremska Zelenika* variety, *Graph 1* 2021 (left) and *Graph 2* 2022 (right)

The rest of 100% of the total individual share is made up of seeds, where no approximate shares were recorded in the examined years. Namely, the highest percentage was recorded in the first year (2021), 3.61%, which also corresponded to the number of seeds in 100 berries, which was 204. In the second year, the percentage of the seeds was much lower amounting to 1.31%, which also corresponded to the number of seeds in 100 berries, being 74.

The results of the chemical composition of the grape must

The results of the analysis of must made of the grapes of the *Sremska Zelenika* variety are presented in Table 3. The sugar content in the grape must (%) was in a narrow range from 20.3% in the first year to 19.3% in the second year. The average sugar content was generally quite high, compared to earlier research, amounting to 19.8%. Namely, the average sugar content recorded in the period 1981–1998 in the same Fruška Gora sub-region (in the same varieties collection), in vineyards with the same training system, was 15.4% (Cindrić et al., 2000). However, one should keep in mind that neither in this nor in previous research there was yield reduction, i.e. reduction of grapes of this high-yielding variety. The results of the research on the chemical composition are more broadly correlated with the achievement of phenological maturity when the average harvest time of the tested variety was September 30th, compared to the earlier research by Cindrić et al. (2000) when the average harvest date was October 7th.

The data obtained from a survey of several grape growers on the sugar content in the must made of the *Sremska Zelenika* variety show similar values compared to this experimental research. Namely, the grape growers in several vineyards in the Fruška Gora sub-region had 19% and 20% sugar in 2021, and 20% in 2022. In the area of the Palić wine-growing sub-region, the average sugar content in the grape musts in 2021 and 2022 was 20%.

Table 3. Chemical values of the grape must made of the *Sremska Zelenika* variety

Year	Sugar (%)	Total acids (g/l)	pH
2021	20.3	7.5	3.11
2022	19.3	6.0	3.43
Average	19.8	6.75	3.27

The results of the content of total acids (g/l) showed this parameter was 7.5 g/l in the first year, and 6.0 g/l in the second year of the research. The average content of total acids for the examined variety was 6.75 (g/l) (Table 3). The results of the content of total acids in this research differed from earlier research within the same facility when the average content of acids was 12.3 g/l (Cindrić et al., 2000). Without going into detailed studies of climatic and other conditions that led to a discrepancy in the obtained data, these results can lead to the conclusion that climate changes with increasing temperatures can positively affect the expression of this quality parameter of grape must.

The experimental grape musts in this two-year research had approximately similar pH values. The average pH was higher in the first year (3.43), while in the second year, the pH was 3.11. The average pH value for both years was 3.27 (Table 3).

CONCLUSION

Based on the research on certain production and technological characteristics of the minor grapevine variety *Sremska Zelenika* without yield reduction measures, it can be concluded that there are some differences between the years of research in terms of the mechanical composition of the clusters and berries, i.e. the structural indicators of the clusters and berries, as well as the chemical composition in the grape must.

Analyzing the results, the average value of the cluster mass (g) of the *Sremska Zelenika* variety for both years was 266.5 g, which is higher than it was recorded in earlier research conducted in the same vineyard in Fruška Gora sub-region.

The chemical indicators of the grape must exhibited a lot of variability between years. The average sugar content was generally high and amounted to 19.8%. The average content of sugar in the grape must in this research is significantly higher than that the same parameter determined in the earlier research in the same locality. The average content of total acids in the grape must was 6.75 (g/l), which is much lower than the values of this parameter found in earlier research in the Fruška Gora sub-region. The experimental grape musts used in this two-year study had approximately similar results, with the average pH of 3.27.

Based on the above-mentioned, it can be concluded that the minor grapevine variety *Sremska Zelenika*, in addition to its high cluster mass, exhibited somewhat improved individual production and technological characteristics under the conditions of the Fruška Gora sub-region (Srem wine-growing region) compared to the results of earlier research. For this reason, this variety deserves further, more detailed research, as well as the possibility for establishing new vine plantations in selected localities with suitable ecological conditions for this variety, all with the reduction of grapes in order to improve the quality of grapes and eventually wine.

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ПРОИЗВОДНЕ И ТЕХНОЛОШКЕ КАРАКТЕРИСТИКЕ МИНОРНЕ СОРТЕ ВИНОВЕ ЛОЗЕ *СРЕМСКА ЗЕЛЕНИКА*

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РЕЗИМЕ: Минорне сорте винове лозе представљају огромну вредност у погледу културног наслеђа, диверсификације производа веће економске вредности и прилагођавања условима животне средине. У овом раду су приказани резултати истраживања појединих производних и технолошких карактеристика (механички састав грозда и бобица, као и хемијски састав шире) локалне, односно минорне беле винске сорте *сремска зеленика* испитиване у агроеколошким условима Фрушкогорског виногорја (Сремски виноградарски рејон) на локалитету Сремски Карловци. У оквиру двогодишњих истраживања (2020. и 2021), без редукције приноса утврђено је да је просечна маса грозда била 266,5 грама. Просечан садржај шећера у шири износио је 19,8%, просечан садржај укупних киселина био је 6,75 грама/литри, док је просечна pH шире од грожђа испитиване сорте била 3,27. Упоређујући поједине утврђене карактеристике са ранијим истраживањима на истом локалитету, закључује се да је минорна сорта *сремска зеленика* показала боље производне и технолошке карактеристике у односу на резултате тих ранијих истраживања.

КЉУЧНЕ РЕЧИ: *сремска зеленика*, Фрушкогорско виногорје, производне и технолошке карактеристике

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ORIGINAL ARTICLE

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HOUSEHOLD CHEMICALS AS POSSIBLE CAUSES OF POISONING AND ENVIRONMENTAL POLLUTION

ABSTRACT: Nowadays, in order to maintain cleanliness in our houses, as well as to maintain personal hygiene, numerous chemical agents are used every day. Dishwashing detergents are used the most, followed by laundry detergents, various soaps and hair shampoos, and not infrequently descaling agents, for cleaning ovens, unclogging drainage and sewage pipes, neutralizing unpleasant odours, etc. Although their number is growing day by day, most people do not realize the consequences, or at least not enough, and especially not about their potentially toxic effects. In fact, a great deal of the population do not consider household chemicals a particular problem, which is true, if they are used in the prescribed manner. However, the fact that these agents can cause harmful effects in humans and animals, and even participate in environmental pollution, is more than a sufficient reason to pay more attention to these agents, that is, to talk about them more and obtain more information for that matter. Particularly since there are authors who believe that some of the mentioned agents can even cause cancer, asthma or birth defects, i.e. infertility. Household chemicals can be divided in several ways, and one of the classifications is the one made according to the place of use. According to this division, the products we use every day at home can be roughly divided into those used for cleaning and maintaining hygiene in kitchens, used for the same purposes in bathrooms (including those used for personal hygiene), and the products used in rooms and on terraces (flower protection).

KEYWORDS: household chemicals, personal hygiene, detergents, soaps, hair shampoos, toxic effects, environmental pollution

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INTRODUCTION

Nowadays, a large number of chemicals are used in communal hygiene, human and veterinary medicine, as well as in agriculture, all over the world. It is safe to say that there is almost no branch of human activity where these tools have not found their application. The largest number of these chemicals are pesticides, i.e. biocides, which are used to destroy microorganisms (disinfection), insects (disinsection) and harmful rodents (de-ratization), both in rooms where people live and where animals are kept, and in the fields, to protect plants from the same pests (Ćupić, 2012).

To be more precise, due to the wide and often irrational or even application which is not in accordance with the Instructions, more and more attention is paid to these products. The firts reason is that they can have a harmful effect, that is, cause certain unwanted or toxic effects, both in humans and in the environment (Ćupić et al., 2002; 2018; Baird, 1999; Crosby, 1998; Đarmati et al., 2007; Hodolić et al., 2009).

A certain number of chemicals (pesticides, biocides and other substances) are also used for cleaning, i.e. maintaining hygiene in our houses, i.e. apartments, or for maintaining personal hygiene. These are various detergents, products for removing limescale, for cleaning ovens, mixed in soaps, hair shampoos or toothpastes, as well as those for neutralizing unpleasant odours, unclogging drainage pipes and others. They may also contain substances harmful to people and the environment. That is why it is very important to be more careful about what is brought into apartments or houses and stored or used in them. The goal of this work is to draw attention to these products, ie. to warn people about possible harmful effects. This becomes even more important, if it is taken into account that people spend over 70% of their time in houses, and especially in apartments (and higher floors) (Pejčić, 2016; Plavšić, 2009; Helmenstine; Belobaba, 2011; Milutinović, 2011; Pejčić, 2011; Pejčić, 2012).

It has been proved that they are only thought about, unfortunately, when an accident happens. In fact, most people do not consider household chemicals to be a particular problem, which is, of course, true, if they are used in the prescribed manner. Namely, surveys have shown that only 3% of the population carefully read the composition of purchased chemicals. Perhaps this is one of the reasons why these chemicals are not talked about enough. However, it should be pointed out that in recent times more and more attention has been paid to the various chemicals that surround us, and therefore also to those found in the house, and there are more and more of them. This is all the more so, because there are more and more people who believe that these products can cause cancer, asthma, infertility or birth defects. Hardly a day goes by that some "new" product for maintaining hygiene at home or for personal hygiene is not advertised on television. Since they can (as already said) have a harmful effect, they should be treated sensibly, that is, with a certain amount of attention. This implies that when using various chemicals in the house, at least the necessary products of personal protection, glovesm ought to be worn. In additon, when talking about these products and their possible harmful effects, it should be pointed out that of all the household members, children represent the most vulnerable category. This applies especially to children up to four years of age, because they are still not able to understand all the dangers and they are very curious and want to know every part of the house. That is why it is qute a responsibility for the elderly not to be careless when using various chemicals and to handle them properly. This implies not only the correct use, but also a whole series of measures related to the storage of chemicals in the house. It would be best if they were kept (if possible) under lock and key, i.e. out of the sight and reach of children, because unfortunately poisoning of children happens almost every day. Cases of drinking dishwashing detergent, swallowing washing powder, bleach, or products used after shaving have been recorded. Unfortunately, these poisonings are increasing and it can be said that they make up a high percentage of the total number of poisonings in people in general (Pejčić, 2016; Plavšić, 2009).

All the listed chemicals, potential causes of poisoning, can be roughly divided into those that are used to maintain hygiene in houses, and those that we use to maintain personal hygiene.

Products intended for maintaining hygiene in houses could be divided in several categories, one of them being – according to the place of use. Hence, the chemicals are divided as follows:

a) *maintaining hygiene in kitchens* (detergents for hand washing dishes, machine washing dishes, cleaning ovens or grills, products for beautifying or caring for crockery and cuttlery (shine), unclogging drainage pipes from sinks, products for removing limescale from taps and sinks, for cleaning, sterilization and maintenance of surfaces, etc.

b) *maintaining hygiene in bathrooms* (soaps, shampoos, balms, foams, descalers from taps, sinks and bathtubs, laundry detergents, stain removal from laundry and other items, air fresheners, toothpaste, mouth disinfectants), nail and hair polish, hair dyes, shaving and aftershaving products and products for unclogging drainage pipes from sinks and bathtubs or sewage pipes, etc.),

c) *maintaining hygiene in the rooms* (floor cleaners – parquet, laminate, for cleaning stains from furniture and polishing furniture, cleaning and washing carpets, armchairs or mattresses), as well as those used for the care and maintenance of flowers.

Quite expectedly, personal hygiene products and cosmetics are also kept in the bathrooms (Pejčić, 2016; Plavšić, 2009).

AGENTS FOR MAINTAINING HYGIENE IN KITCHEN

Dishwashing detergents

After each meal, which is done several times during the day, dirty dishes and utensils should be washed (by hand or by machine). Pots and pans are a major problem, especially if burnt during the food preparation. For this purpose, numerous products are advertised on television every day, where their effectiveness in removing dirty deposits from dishes is emphasized to the maximum. In other words, all the problems with dirty dishes will be solved if one one buys one of these products, which certainly is not the case. Namely, it has to be well understood that the more effective a product is, usually the more toxic it is and more dangerous for the person who washes the dishes. It would be best if washing could only be done with clean water, but this is unfortunately only possible in a limited number of cases. Washing greasy and burnt, and sometimes unpleasantly smelling pots or pans cannot be effectively washed with water alone, but various chemicals must be used, i.e. detergents. Our ancestors used sand for these purposes once upon a time. There is no need to return to these (traditional) ways of washing, but we need to find out what kind of danger new modern washing agents bring us (TigerDoor, 2023; Pejčić, 2016; Plavšić, 2009).

Therefore, in accordance with the degree of soiling, various chemical agents are used for manual washing. When washing in a dishwasher, people will use it according to the advice, i.e. the instructions for use. One simply needs to put all the necessary detergents and other agents, such as those for shine and others that improve the washing efficiency, i.e. prevent the accumulation of limescale, and after that turn on the machine. This is an advantage over hand washing (Pejčić, 2016; Plavšić, 2009).

Detergents from the group of nonionic surfactants, or detergents, are most often used for washing dishes. In addition to the disinfecting effect, they are also used because they dissolve, i.e. they dissolve fats or other organic substances and in this way it is easier to remove impurities from dishes or other objects. In products used for manual dishwashing, in addition to nonionic surfactants, there are also anionic surfactants, and they may also contain preservatives (methylisothiazolinone or phenoxyethanol, i.e. paraben), and the monoterpenes citronellol and limonene (Plavšič, 2009; Vapa, 2016; Samardžić, 2020).

These products or surfactants, in their pure state*, cause severe eye irritation and are very toxic to aquatic life with long-term consequences* (*Sl. glasnik RS*, 2010). However, it can be found in the literature that the above-mentioned preservatives (methylisothiazolinone or phenoxyethanol) also have toxic effects. Thus, for methylisothiazolinone it is stated *that it can cause allergic reactions in humans,* while *for phenoxyethanol there are data that it is a potential carcinogen* (*Sl. glasnik RS*, 2010). Furthermore, the tests have shown that anionic surfactants in zebrafish can cause serious histological degenerative and necrotic changes in the gills, and a change in behaviour (hyperactivity) has been observed (Samardžić, 2020).

Detergents used for household cleaning are toxic to aquatic organisms at concentrations of 0.07 mg/L to 35.4 mg/L. Anionic surfactants and bleaching agents have toxic effects at concentrations of 0.0025 ± 300 mg/L to 0.3 ± 200 mg/L and 5 ± 9 mg/L to 4.6 ± 226 mg/L (Ankley and Burkhard, 1992).

Bearing in mind that the mentioned substances are found in the products in a concentration of less than 10%, i.e. 5%, and that they are diluted several times immediately before application, then it is unlikely that in such a state they will have a harmful effect, both on people and and the environment. In addition, as such, they can be discharged into the sewer without restriction. It could be said that their only problem is that they create foam. In a technical sense, creating foam is not a problem, because it helps to remove impurities. However, it should be pointed out that these agents (due to the formation of foam) are potentially dangerous if swallowed. There were even cases of suffocation of children with foam, which rose from the stomach to the mouth after swallowing. Although one should think about these things and always prevent children from coming into contact with such products, one should know that in such cases silicone compounds can be used, which have the power to break the foam (Vapa, 2016; Plavšić, 2009).

For heavily soiled dishes, other chemical agents can be used, such as: hydroxides of alkali metals, quaternary ammonium compounds, sodium salt of ethylene-diamino-tetraacetic acid, i.e. ethylene diamino tetra acetate (EDTA), then various detergents, and often agents for disinfection. Alkaline hydroxides are known to be *corrosive substances, EDTA is harmful if swallowed and causes eye irritation, quaternary ammonium amines are corrosive and very toxic to organisms living in water and detergents with the property of irritating or irritating the eyes, very often they are toxic to organisms that live in water and have a long-term harmful effect in water* (*Sl. glasnik RS*, 2010). Therefore, the final product is usually corrosive, and in the best case it is toxic to aquatic organisms. These dangerous properties certainly cannot be easily ignored. Therefore, when washing and using these products, it is mandatory to wear prescribed personal protective equipment. Better to say, without using suitable gloves (for example, made of nitrile or butyl rubber) it is better not to do anything, and it is not out of place to wear a suitable apron and even glasses to protect the eyes. If used unprofessionally, these agents can cause burns to the skin or eyes, and swallowing is a particular problem. If something bad happens to you or your child, it is necessary to provide first aid, which consists in the application of means or drugs to relieve burns, redness and pain (anti-inflammatory drugs, analgesics) (Samardžić, 2020; Vapa, 2016; Plavšić, 2009).

Means for machine washing are similar to those used for hand washing dishes, but as a rule they also contain disinfectants. They can be found in circulation in the form of powder, solution or balls. For these purposes, numerous agents are used, which may contain oxygen-based bleaches, then nonionic detergents, polycarboxylates and phosphonates, enzymes (subtilisin, amylase), which help the action of nonionic detergents, and agents for shine, as well as disinfection (for example sodium hypochlorite). *All of them cause skin and eye irritation, and can act as sensitizers* (*Sl. glasnik RS*, 2010).

Nowadays, there are numerous products that are used to sterilize dishes. For example, triclosan or 2,4,4-trichloro-2-hydroxydiphenyl ether is often used for these purposes (especially in Western countries). Such preparations, in addition to disinfectants, as a rule also contain some bases (for example, potassium hydroxide, sodium carbonate, etc.), and various other additives, if at the same time they serve for washing (for example, nonionic surfactants, disodium metasilicate and/or tetrasodium EDTA). Most certainly, the worst component is a base, but active chlorine is not completely negligible either. There will certainly be more to say about this with products for disinfection in bathrooms. *Such produscts, i.e. preparations that contain them, in contact with the skin, can cause mild burns, with a burning sensation or pain, and later infections are common. If they get into the eyes, they can cause damage to the cornea. In the case of inhalation of chlorine, symptoms such as coughing and shortness of breath may occur, which may persist for a day or two after exposure. Swallowing such substances is very dangerous and severe damage to the upper parts of the digestive system is possible, even with the occurrence of bleeding. In addition, these agents are dangerous for aquatic organisms with long-term consequences.* In any case, after swallowing such a chemical, a doctor's help must be sought (*Sl. glasnik RS*, 2010; Majnhen; Pettersson i sar., 2000; Samardžić, 2020).

In recent years, considerable attention has been focused on detergents that are widely used in daily personal hygiene and household products, as well as in various industrial processes. As a result, large quantities of products are usually discharged into wastewater treatment plants or directly into the aquatic environment in areas where there is no way to treat wastewater (Profepa, 2002; Mei-Hui, 2008).

Means for cleaning and washing ovens and grills

Furthermore, oven and (to a lesser extent) grill cleaning agents are used in kitchens. Although a high temperature can be used for these purposes, today quite strong cleaning agents, which contain mainly mixtures of strong bases (for example, sodium hydroxide) and surfactants (mostly non-ionic) are used most often. *All of them are classified as substances that cause severe skin burns and eye damage* (*Sl. glasnik RS*, 2010; Samardžić, 2020).

They are available in the market, usually in the form of a spray, because they are easier to apply to all parts of the oven. During this work, hands, eyes and respiratory tract should be protected equally. Although their application creates a stable foam, they still irritate the respiratory tract, so coughing is not uncommon. Although we can say with high probability that there is no danger of swallowing a chemical from a spray bottle, nothing is ever ruled out. *In any case, with careless and improper handling, these agents can cause very painful burns on the skin with the appearance of vesicles, and due to possible splashing in the eyes, even vision can be lost.* Since housewives most often do not have masks or any other equipment to protect the respiratory tract, it is recommended to ensure good ventilation of the room during the application and that the application lasts as short as possible. In any case, when applying, one must wear gloves (for example, surgical ones) and (if possible) some glasses, in order to protect themselves against possible splashing of the agent in the eyes. These types of products are very dangerous and must be stored very carefully, away from children. Therefore, such changes require mandatory treatment. *If these agents are swallowed (which is unlikely), severe damage to the mucous membranes of the oral cavity and esophagus is possible. Bleeding cannot be ruled out, but esophageal ruptures are unlikely. There is also a possibility of damage to internal organs, such as the kidneys and liver.* Poisoning caused by the ingestion of alkali (with this warning label) is very serious, and the treatment usually takes a long time (*Sl. glasnik RS*, 2010; Samardžić, 2020; Plavšić, 2009; *Sl. glasnik RS*, 2009–2023).

Disinfectants

In addition to the dishes and the oven, every housewife strives to keep her kitchen as a whole spotlessly clean from dirt and all living organisms, especially bacteria and mold. This especially applies to surfaces where food is kept, that is, where food is eaten. A large number of disinfectants can be used for these purposes, out of which those based on active chlorine should be mentioned in particular. In addition to preparations containing sodium hypochlorite, trichloroisocyanuric acid and representatives of cationic detergents, such as quaternary ammonium compounds (cetrimonium bromide), are often used. Sometimes there are ready-made solutions for use (1% solution of some quaternary ammonium compound). Given that the other two are much more related to application in bathrooms than in the kitchen, here we will only talk about trichloroisocyanuric acid. It is known to be very widely used as an effective disinfectant, and it is available in the market in its pure state, in the form of a solution or in solid form (tablets). It is very dangerous in its pure form. *In contact with flammable material, it can cause a fire, it is harmful if swallowed, in contact with acids it releases poisonous chlorine gas, it leads to strong irritation of the eyes and respiratory organs, and it is very toxic to organisms living in water, with long-term consequences.* You should not be too afraid because of all the abovementioned, because it is found in preparations in low concentrations, in which many dangerous properties are lost and most often remains this one (*in contact with acids it releases poisonous chlorine gas, it is irritating to the eyes and mucous membranes of the respiratory organs, and is toxic to aquatic organisms with long-term harmful consequences*). Since it is difficult to find in the household in its pure state, it is unlikely to cause the effects mentioned above. In any case, protective gloves and possibly goggles should be worn when applying this flat surface disinfectant. Swallowing, in the worst case in children, can cause irritation of mucous membranes and vomiting (*Sl. glasnik RS*, 2010; Plavšić, 2009; Samardžić, 2020; *Bezbednosni list*, 2006).

Descaling agents

In the kitchens (as well as in the bathrooms), various chemicals are used to remove scale, which forms primarily on faucets and sinks. Since it creates the impression that the mentioned items are dirty, housewives try to restore their shine by using certain chemical agents. Nowadays, there are a large number of preparations on the market (most often in the form of sprays) that are used to remove limescale. These agents usually contain some acid (for example, phosphoric, orthophosphoric, sulfamic), surfactants (anionic and/or nonionic detergents), and other substances, such as glutaraldehyde (for disinfection) or quaternary ammonium compounds. Some of the components, such as *glutaraldehyde or glutaral, are quite toxic in their pure state (toxic if swallowed, causes severe skin burns, can cause allergic reactions on the skin, toxic if inhaled and very toxic to aquatic organisms).* However, its concentration in preparations is usually low and amounts to about 0.3%. At this concentration, glutaraldehyde loses most of its dangerous properties (*Sl. glasnik RS*, 2010; Plavšić, 2009; Samardžić, 2020).

Phosphoric acid is also available in traffic in concentrations below 15%, which are not dangerous. Out of several dangerous properties, only the property of irritation remains. Surfactants are also found in concentrations below 10%, *where they are ecotoxic and irritating to the eyes.* In the end, the products containing the mentioned substances could be classified as *those that irritate the eyes, respiratory organs and skin, and possibly have a harmful effect on organisms in the water with long-term consequences.* This means that we can use them with minimal precautions. However, when applying them, it is recommended to wear protective gloves and goggles. Bearing in mind that they are available in the form of a spray, it is assumed that there are few opportunities for them to be swallowed, except perhaps by children. In any case, care should be taken not to inhale aerosol particles, as they could cause irritation of the mucous membranes in the respiratory tract. These tools should be kept out of sight and reach of children (*Sl. glasnik RS*, 2010; Plavšić, 2009; Samardžić, 2020).

Means for unclogging drainage pipes

Drainage pipes are also a significant problem in homes, both in kitchens and in bathrooms. Sink, washstand and bathtub pipes are most often blocked, and the cases of blocked drainage pipes and toilet bowls are not rare. Nowadays, unfortunately, we often witness that individual tenants behave carelessly and irresponsibly, stuffing (throwing) everything into the unspecified drainage pipes, which then need to be unclogged. In the apartments above the place where the blockage occurred, waste water and feces often appear. In such situations, a person tries in every possible way to make clogged pipes passable. Some first try to look at and clean the siphon with the hope that this will eliminate the problem, but the pipes can also get clogged under the siphon. In addition to mechanical means (vacuum tires, hot water, cables or blowing under high pressure), various chemicals can also be used to unclog pipes (Samardžić, 2020; Pejčić, 2016).

There are two basic possibilities, to apply produscts that act quickly and strongly or those that act gently and slowly. Corrosive chemicals are used for the first purpose, and biological agents in the form of enzymatic cleaners for the second. The latter will not be discussed here, because they are almost not on the market, and besides, they are not toxicologically significant. Therefore, we will talk about strong corrosive agents, which of all common household agents are among the most aggressive and therefore toxicologically significant. As such, they can cause numerous problems (Pejčić, 2016; Plavšić, 2009).

Strong bases or strong acids are most often used for these purposes. As for the acids, some housewives most often use technical sulfuric acid, as well as hydrochloric acid. As bases, a mixture of sodium hydroxide and sodium hypochlorite is usually used. By applying this mixture, the hydrolysis and oxidation of impurities in the drainage pipes takes place at the same time. All of these agents work quite effectively, but it should be taken into consideration that they (primarily acids) can cause great damage to drainage pipes. In fact, after the application of these agents, especially if it is frequent, cracks can appear on the drainage pipes, and as a result, flooding and leaking sewage to the neighbours below. This was especially a problem in apartments with old lead pipes. In medical terms, these agents are much more dangerous than those used to clean ovens and grills. It is safe to say that all the consequences that can be caused by oven cleaners can also be caused by these, only the symptoms are even more pronounced. These agents are very poisonous, and *after swallowing they irritate the mucous membrane, causing pain in the mouth, throat and stomach, with diarrhea and bleeding. Moreover, in contact with the skin, they cause burns and wounds, which are difficult to heal. After inhalation of vapours and gases, they act as irritants, causing coughing and difficulty breathing, dizziness and headache* (Pejčić, 2016).

Products for washing and cleaning floors and kitchen furniture

To maintain hygiene in kitchens, products for washing and cleaning floors are also used. Namely, the floors should be washed regularly, because during the cooking process, not only the products of the vapours reach the floor, but also various parts of the food. For washing floors, mostly mild products are used, i.e. detergents, practically without any danger signs or warning labels. If this work is done while kneeling and holding a wet cloth soaked in a detergent solution in the hand, then it is necessary to use gloves. However, today there is also special equipment for washing floors, which makes it possible to avoid having to come into contact with the detergent with bare hands. Even if you touch it, there would be no health consequences, which certainly does not mean that it is recommended to work without protective gloves. In addition, it should be noted that swallowing such detergents, which produce weak and unstable foams, should not be a problem. *Only nausea and possibly vomiting can be expected* (Pejčić, 2016; Plavšić, 2009).

However, it should be noted that today on the market you can find so-called universal products, used for washing floors, regardless of whether they are made of glass, ceramic, wood or metal. They usually contain less than 5% of anionic detergents and the same amount of nonionic detergents, and acids (benzenesulfonic acid), secondary alkyl alcohols, ethoxylated sulfates and sodium salt. Even though they are diluted 1:10 immediately before application, it is characteristic for them to *cause severe skin irritation and severe eye damage* (*Sl. glasnik RS*, 2010; Pejčić, 2016; Plavšić, 2009).

Kitchen furniture should also be maintained, primarily by dusting or removing greasy stains or disinfecting. Since the kitchen furniture today is made of chipboard or mediapan, their outer surfaces are covered with resistant varnishes. Therefore, no dangerous chemicals are needed to maintain their cleanliness. Mild detergent for occasional washing or even plain water is sufficient. In addition, the above-mentioned material is already protected against various insects during industrial production, so you should not use anti-fungal or weevil agents. For this reason, there is no fear of chemicals when maintaining kitchen furniture (Pejčić, 2016; Plavšić, 2009).

External parasites, i.e. insects, such as ants, flies and spiders remain in the kitchens. Although these parasites are practically not dangerous and do not transmit infectious diseases, people do not like to see them in their apartments, so they often use various pesticides, i.e. biocides, to control them. For these purposes, preparations based on pyrethroids (for example, permethrin) are most often used in various forms, most often in the form of powder and spray. It is characteristic of these agents that they are *harmful if they are swallowed, that they can cause allergic reactions on the skin, that they are harmful if they are inhaled, and that they are very toxic to aquatic life with long-term consequences* (*Sl. glasnik RS*, 2010; Plavšić, 2009).

PRODUCTS FOR MAINTENANCE OF HYGIENE IN THE BATHROOM

In addition to the fact that the hygiene and beauty of the body is maintained in the bathroom, there is traditionally a washing machine in them, and therefore also detergents, as well as other means, which are used for washing, i.e. maintaining personal hygiene. It is safe to say that the bathroom, of all places in the apartment, contains the largest number of various chemicals. When it comes to maintaining the hygiene of the human body, it is logical that for these purposes, dangerous agents, which harm the human body, must not be used. These are primarily various soaps, foams, shampoos, aftershave lotions, hairspray, nail polish, nail polish removers, etc. Furthermore, part of the products from the kitchen are used here, such as disinfectants, products for dissolving or dissolving limescale or products for cleaning drainage pipes, thus it is not necessary to mantion them again (Pejčić, 2016; Plavšić, 2009).

Products for maintaining personal hygiene

Washing and body care products must have some special properties. First of all, they must not cause any harmful effects, i.e. have a harmful effect on the human organism or the animals organisms, because the bathroom is usually used for washing pets, i.e. dogs. The main ingredients of solid soaps, gels or sprays are surfactants (salts of fatty acids such as lauric acid, then alkyl sulfonates, various ethoxylated long-chain amines, amides such as cocoamide, etc.). One of the well-known soaps, which is currently on our market, has a rather complex composition and in a certain mass proportion contains: sodium lauryl isethionate, stearic acid, sodium palmitate, methyl propionate, lauric acid, sodium isethionate, sodium -stearate, glycerin, propylene glycol, sodium chloride, tetrasodium EDTA, zinc oxide, butyl-phenyl and limonene. The basic role of soap, i.e. the substances contained in it, is to dissolve and bind impurities, thereby enabling their dispersion in water. Mostly, these products are risk-free or represent a very small or low risk to health (Pejčić, 2016; Plavšić, 2009).

As mentioned, in addition to the basic soaps, there are also a large number of substances that help or facilitate their action (for example, glycerin), then those that additionally disinfect the skin (various disinfectants), protect the skin epithelium, etc. None of the listed agents or ingredients can be harmful to human health, even though it will only come into contact with the skin and mucous membranes or it will be swallowed, for example. In sensitive people, *it can happen that, for example, some shampoo causes a slight irritation of the eye mucosa, but this is a very short-term effect, which disappears very quickly after the end of exposure.* It is really rare that someone has been poisoned by soap or hair shampoo, regardless of the strength, although nowadays, in addition to mild shampoos, there are shampoos on the market that are used, for example, to remove dandruff. They can *certainly cause stronger irritation of the mucous membrane of the eyes* (Pejčić, 2016; Plavšić, 2009).

Therefore, when it comes to such products and their composition, certain questions are always asked. There was a lot of controversy about the disinfectant triclosan. This disinfectant is widely used, and in a home it can be found in detergents, deodorants, toothpastes, various other cosmetic preparations, etc. It has already been discussed. Therefore, as a pure substance, it is considered harmful to human health and is ecotoxic, *i.e. it causes skin irritation, leads to severe irritation of the mucous membrane of the eyes, and is very toxic to aquatic life with long-term consequences.* However, it is applied in very low concentrations, thus some of the mentioned harmful effects are lost. Therefore, in disinfectants, in which it is present in a concentration of 1%, the only property of weak ecotoxicity is retained *(harmful to aquatic organisms and can cause long-term adverse effects in the aquatic environment).* In the concentrations in which it is found in soaps, shampoos and similar products, it no longer has any harmful effects on human health or the environment, and as such it is widely used as a completely harmless ingredient in cosmetic preparations (Pejčić, 2016; Plavšić, 2009).

However, data appeared in the literature that it is structurally similar to dangerous dioxins. Special attention was paid to its harmful effects on hormone synthesis after long-term exposure. Some studies have shown that it is very easily deposited in fat, that is, adipose tissue, which can cause numerous disorders in the long term, such as diseases of the liver, kidneys, brain, sexual organs, etc. Naturally, after such information, people began to fear such a substance and began to avoid preparations containing triclosan. However, none of the aforementioned claims have ever been officially confirmed. Its main property is chemical instability and decomposition with the release of active chlorine. That is why it is used as a disinfectant because it releases chlorine, which has an antibacterial effect. Hence, how should people behave and should they always accept the claims of scientists a priori? That is why one should be very careful when analyzing scientific works. This especially applies to those papers in which data on the harmful effects of certain chemicals are presented. In addition, in our country, triclosan is on the list of classified and marked substances. According to the same list, this substance in its pure state irritates the skin and mucous membranes, and is very toxic to aquatic life with long-term consequences (*Sl. glasnik RS*, 2010; Plavšić, 2009).

In addition to soap and shampoo, toothpastes are often used to maintain personal hygiene, i.e. hygiene of the oral cavity. Depending on the manufacturer and purpose, they may contain numerous substances. For example, on the box of one of the pastes used on our market, it can be seen that it contains 1.1% sodium salt of fluorophosphate. The second, on the other hand, has a more complex composition and contains a certain mass proportion: sorbitol, glycerin, sodium lauryl sulfate, xanthan gum, sodium fluoride, magnesium sulfate, disodium phosphate, zinc sulfate, sodium sulfate, sodium benzoate, eugenol and limonene. It is particularly emphasized that the amount of sodium fluoride is 1450 ppm. Therefore, both of these pastes contain sodium fluoride, i.e. fluoride, which is added to prevent caries. For the same purpose, sometimes fluoride salts are added to drinking water. In our country, sodium fluoride is on the list of classified and marked substances. According to the same list, *this substance is toxic if swallowed, and leads to irritation of the skin and mucous membranes of the eyes* (*Sl. glasnik RS*, 2010).

However, it should be noted that nowadays there are many people in the world who are against this toothpaste ingredient, because, as already stated, fluoride is a very toxic substance. Suffice to say that it was used as a means of destroying rodents, and there is information that the Nazis used it to sterilize prisoners in the camps, as well as to turn them into “obedient slaves”, i. e. to kill the will to live in them. In addition, fluorine is a key ingredient in some drugs used to treat depression, and it is also found in the nerve agent sarin. It should not be especially mentioned that it is also present in a well-known group of antimicrobial drugs, which are called fluoroquinolones (TransformacijaSvijesti, 2013; Marinković, 2012; Ćupić et al., 2019).

For a nice smell and facial care, men use certain lotions after shaving. In one of them, which is on our market, there is a whole range of ingredients. These are: alcohol, citral, citronellol, coumarin, geraniol, castor oil, limonene, linalool, glycerin, ethylhexylmethoxyricinamate, ethylhexylsalicylate, propylene glycol, benzyl alcohol and benzyl salicylate. Although most of these ingredients are mild in nature, i.e. of natural origin, it is not excluded that they can *cause irritation or* (in a small percentage) an *allergic reaction in people with sensitive skin*. In addition, some of them, such as limonene, are on the list of classified and labeled substances. According to the same list, this substance *causes skin irritation and sensitization, and is very toxic to aquatic life*. Although not on the list, geraniol also *causes skin and eye irritation* (*Sl. glasnik RS*, 2010; Pejčić, 2016).

Furthermore, women use varnish for the care and beauty of their nails, which they occasionally have to remove using organic solvents, such as acetone. Acetone is known *to belong to easily flammable liquids, it leads to strong eye irritation, and it can cause drowsiness and fainting.* In this regard, one should be careful not to get acetone on their hands during use, and of course, when applying it to their nails, they should keep thier hands as far away from their nose as possible, with slower breathing. In addition, care should be taken not to swallow it, and accordingly, it should be kept away from children. Other chemicals, such as various cosmetics, can be found in the bathroom. Although regular control and supervision of these preparations is carried out, some attention (especially when it comes to children) is always welcome (*Sl. glasnik RS*, 2010; Plavšić, 2009; Pejčić, 2016).

Due to the ability to cause a whitening effect, oxidants can also be used for other purposes, primarily for a more beautiful face. As for the hair, they use bleaches similar to those used for linen and are not harmless. Thus, one such product (powder) consists of a mixture of potassium persulfate, ammonium persulfate, sodium persulfate and disodium metasilicate. All these substances are oxidants, with a corrosive effect. Products containing them are classified *as corrosive and oxidizing chemicals with the following hazard notices: can cause or encourage fire, is harmful if swallowed, causes severe burns, as well as allergic skin reactions, eye damage, can cause irritation respiratory organs, if inhaled it can cause allergic reactions, asthma or breathing problems* (*Sl. glasnik RS*, 2010; Plavšić, 2009; Pejčić, 2016).

Based on these data, it can be concluded that these substances are not harmless. Although their toxicity is reduced by dissolving them in water, immediately before treatment, it should be noted that the bleaching solution will have similar properties to the previously mentioned preparation. Therefore, the question arises whether such products can be kept in the house at all. When it comes to children, this preparation presents a similar danger, as a tool for machine washing dishes.

Teeth whitening is done mostly in dental offices and there have almost never been complaints from citizens about the consequences. In Western countries (and there is information here as well), tooth whitening pastes can be bought over the counter, so teeth whitening is done at home on your own, regardless of the fact that there may be minor damage to the gums. In any case, if you buy this product, you should take care of possible ingestion (which is the only way of exposure), so the same product must be kept out of the reach of children. If the child gets to this product and swallows it, it will probably *vomit and have a burning sensation or even pain in the mouth and esophagus, a few days after the incident* (*Sl. glasnik RS*, 2010; Plavšić, 2009).

In conclusion, it can be said that personal hygiene products (primarily soaps and shampoos) do not represent a health problem. Some even claim that they do not have to stay away from children, because children should be treated with the same or similar products. Contact with the skin and mucous membrane of the eyes will generally not cause any consequences, and in case of swallowing, the only thing that can happen is the formation of foam in the digestive tract. However, it should be said that (depending on the type and composition) some shampoos, i.e. soaps, can (at least for a short time) *irritate the mucous membrane of the eyes.* Bearing all this in mind, it can be concluded that children should definitely not be allowed to swallow such products, and if a child happens to swallow a piece of soap, there is no need to panic (Pejčić, 2016; Plavšić, 2009).

Neutralization of unpleasant odours

These agents are mostly used in bathrooms, because quite unpleasant odours can spread from the toilet bowl. In addition, deodorants are also used in other rooms in a home, even in cars. However, when it comes to the bathroom, deodorant manufacturers have gone one step further. Numerous products have been made in various forms, the main purpose of which is to neutralize unpleasant odours from the toilet bowl. In addition, they usually also contain disinfectants, surfactants, agents for dissolving limescale and other additives, which enable the gradual release of active substances, such as various solvents. Odours are usually harmless and do not have a significant impact on the health of people who spend a long time in the bathroom (Pejčić, 2016; Plavšić, 2009).

Most of the active components of the mentioned agents are found in relatively low concentrations, so no harmful effects should be expected. In addition, they are in the toilet bowl, so the water dilutes them and carries them away. One of the more well-known agents, which is found on our market, and is used for the purpose of neutralizing unpleasant odours from the toilet bowl, consists of >30% anionic detergents, 5–15% nonionic surfactants, disinfectants, descaling agents and perfumes (coumarin, limonene). Cheaper disinfectants certainly include formaldehyde and glutaraldehyde, which can also be found in hair shampoos. Both of these substances are toxic and are on the list of classified and labeled substances. According to the same list, formaldehyde *is toxic in contact with the skin, if swallowed and inhaled, causes severe burns and eye damage, skin sensitization, and is a carcinogen.* As for gluaraldehyde, it is stated that it *is toxic if inhaled, in contact with the skin, if swallowed, that it can cause allergic reactions, both in contact with the skin and after inhalation, and that it is toxic to the living world in water.* Phenol is also mentioned, but according to the data we have, it is not used in deodorants for toilet bowls. However, the above-mentioned substances are found in preparations in rather low concentrations, where their dangerous properties are lost (*Sl. glasnik RS*, 2010; Pejčić, 2016; *Sl. Glasnik RS*, 2009–2023).

Chemicals for dissolving scale have already been discussed before and they do not have any significant effects, because all dangerous properties are lost at the concentrations in which they are found in such preparations. However, on the boxes of some of the products used for air freshening, i.e. deodorization in bathrooms and which are placed on the wall, it is written *that* *they are highly flammable and that they have a harmful effect on aquatic life with long-term consequences.* That is why substances to prevent ignition are added to them (Plavšić, 2009; Pejčić, 2016).

One of the agents that certainly deserves attention is dichlorobenzene, which (in the context of detergents and deodorants) is used as a disinfectant. Namely, for a while there was a lot of controversy about dichlorobenzene, which evaporates from solid deodorants or substances such as dimethyl-methylphosphonate. Back in the 1960s, information appeared that this compound is carcinogenic. This problem is best illustrated by the example of different forms of dichlorobenzene (o-, m- and p-), which in the end (even though it is the same compound) still have different toxicological properties. Some authors believe that this compound evaporates relatively poorly from solid substances, and that the accusations of endangering human health do not correspond to the real truth. On the other hand, there are those "scientists" who (based on tests performed on animals, but not on humans) warn that this compound can cause liver and kidney cancer. However, the official data of the EU Commission should be trusted, and as far as can be seen, it has not paid special attention to these substances so far. Otherwise, dichlorobenzene is on the list of classified and labeled substances in the form of 1,2-dichlorobenzene (o-dichlorobenzene) and 1,4-dichlorobenzene (p-dichlorobenzene). According to the same list, 1,2-dichlorobenzene *is harmful if swallowed, causes severe eye and skin irritation, and causes irritation of the respiratory organs.* On the other hand, 1,4-dichlorobenzene states that this form *also causes severe eye irritation*, but (unlike 1,2-dichlorobenzene) *is highly toxic to aquatic life with long-term effects and is suspected to cause until the appearance of cancer* (*Sl. glasnik RS*, 2010; Plavšić, 2009; Pejčić, 2016).

Bearing in mind all of the above, we believe that this last statement should not be an inhibiting factor for further use of deodorant, both in the bathroom or other rooms in the apartment, and on the body itself. In other words, it can be concluded that those who claim that we should not stay for a long time in the bathroom or toilet, where deodorants are used, are not right. However, most authors believe that (in order to maintain personal hygiene) the body should be washed more and various deodorants should be used less to cover up unpleasant odours (Pejčić, 2016; Plavšić, 2009).

Laundry detergents

The washing machine is most often found in the bathroom and it plays an important role in the life of every average citizen. Nowadays, laundry is washed more and more often, because machine washing is, first of all, simple and fast. Better to say, machine washing has become indispensable today, thus housewives very rarely and only in certain situations (for example, when it comes to sensitive fabrics) decide to wash by hand. Needless to say, machine washing requires detergents, and today they are very complex mixtures of different substances, some of which are harmless, and some of which are dangerous for human health or the environment (Pejčić, 2016; Plavšić, 2009; Smulders, 2002).

What is good is the fact that today there is a real competition between manufacturers to put on the market the least harmful products. This is not always possible, because detergents, in addition to harmless enzymes, must also contain various surfactants and bases, but in quantities that do not pose a danger to human health. They also adapt to environmental protection requirements, and recently, for example, they are increasingly removing phosphates from detergents, which are not harmful to human health or the environment. These were normally added to prevent the formation of limescale. However, it was shown that phosphates, as a nutrient, stimulate the growth of some simple organisms (algae) in all waters in which they are found. The creation of algae is an ecological problem that resulted in certain chemical changes, i.e. a decrease in the amount of oxygen in the waters where they were formed, and thus all other disturbances in the aquatic ecosystem. This was the reason that phosphates were for a time banned for use in detergents, both for laundry and dishwashers. Only in the so-called washing powders of the fourth generation, which contained zeolite and organic polycarboxylates, this problem was solved, which means that the goal of preventing the formation of scale in machines and the formation of algae in aquatic ecosystems was simultaneously achieved (Pejčić, 2016; Pfendt, 2009; Plavšić, 2009; Nowack, 2003).

Detergents for machine washing in the market are mainly in the form of powder or liquid, and they contain substances, which generally *have an irritating effect on the mucous membrane of the eyes.* Other properties are not worrisome, because manufacturers try to keep the concentrations of dangerous substances below those at which harmful effects can occur (Plavšić, 2009; *Sl. glasnik RS*, 2010).

One of the products on our market in liquid form, intended for washing and softening laundry, contains the following substances: anionic detergents (5–15%), nonionic detergents (less than 5%), and soap. polycarboxylates, phosphonates, enzymes, butyl-phenyl-methyl-propionate, citronellol, geraniol, methylisothiazolinone, etc. Some of them have already been discussed, with the products used for machine dishwashing. Furhermore, the label of this product states that it *causes serious eye irritation* (Pejčić, 2016; Plavšić, 2009; *Sl. glasnik RS*, 2010).

In addition, when it comes to the quality of these products, it should also be mentioned that there are many who claim that the products of the same name in the West and in our country (one can say the whole region) are not of the same composition or quality, in other words that they are sent to us as waste or trash. In any case, when applying detergents, i.e. putting them in the machine, it is not out of place to put on protective gloves. Regardless of all the advertisements about the effectiveness of certain detergents, unfortunately we have witnessed that not all stains can be removed from clothes by machine washing, but some other products must be used. Suits and dresses are usually dry-cleaned, but even in this way not all stains can be removed from clothes (Plavšić, 2009).

There are basically two approaches to stain removal. One is to *bleach the clothes* and the other is to use appropriate *organic solvents.*

Bleaching is an older method of cleaning, regardless of how the job was done. In the old days, people exposed their clothes to the sun, and in that way they dried, disinfected and bleached them at the same time. Today, this is done with chemicals, predominantly with oxidants. Most often, agents based on sodium chlorite, sodium percarbonate, hydrogen peroxide, peracetic acid and similar chemicals are used. Oxidants carry out oxidation of the colours on the fabric and in this way their properties are lost, i.e. the colours on the canvas fade. They are suitable for many colours insoluble in organic solvents (Pejčić, 2016; Plavšić, 2009).

For hygienic and cosmetic purposes, hydrogen peroxide is used in diluted form (3%, 6% and 12%) as an antiseptic. Notably, hydrogen peroxide is on the list of classified substances and has been assigned danger notification marks, which shows that this substance *can cause a fire or explosion, that it is a strong oxidizing agent, that it is harmful if if swallowed, causes severe skin burns and eye damage, and is harmful if inhaled.* All this points to the fact that hydrogen peroxide is not a harmless chemical. However, in concentrations below 5%, a large number of the mentioned effects are lost (remains harmful if swallowed, irritation of the mucous membrane of the eyes). However, preparations containing hydrogen peroxide should be handled carefully and gloves made of nitrile or butyl rubber must be used (*Sl. glasnik RS*, 2010; Pejčić, 2016; Plavšić, 2009).

Other oxidants are similar in their toxic properties. Thus, sodium percarbonate in its pure state acts *as an oxidant and is harmful, i.e. in contact with flammable material it can cause a fire, it is harmful if swallowed, causes skin irritation and leads to strong eye irritation,* but it is used in significantly higher concentrations. rather than hydrogen peroxide (*Sl. glasnik RS*, 2010; Pejčić, 2016; Plavšić, 2009).

Peracetic acid is used for disinfection and is on the list of classified substances. According to the same, this acid *is inflammable in liquid or vapor form, heating can lead to fire, is harmful if swallowed or inhaled, is harmful in contact with the skin, causes severe skin burns and eye damage and is very toxic to aquatic life with long-term effects.* In the concentrations in which it is used, it remains capable of causing *irritation of the skin, mucous membranes of the eyes and respiratory organs* (Pejčić, 2016; Plavšić, 2009; *Sl. glasnik RS*, 2010).

Therefore, all these oxidants, in the concentrations in which they are applied, in the worst case are harmful after ingestion, and all of them irritate the eyes and the skin. It is actually not as dangerous as it might appear to be, but they should still be kept away from children. *After swallowing, burning or pain in the upper part of the digestive tract can be expected, along with nausea and vomiting. In higher concentrations at the place of spraying, they can cause depigmentation of the skin, and also cause minor or major damage to the eye mucosa (tears, redness, burning or even pain).* If there is contact with the skin or eyes, the usual decontamination should be carried out (rinsing with a large amount of water), and only then seek medical help (Plavšić, 2009; *Sl. glasnik RS*, 2010).

As for spots or stains, especially greasy ones, it is better to remove them with chemicals, i.e. organic solvents. Nowadays, there are numerous products that are intended for dry cleaning, that is, that are used in dry cleaning. In fact, all those stains for which there is no guarantee that they will be removed in the usual washing process, they must first be treated with these products. Similar products are used in the household, but not all of them are used professionally. There are currently several (more or less effective) stain removers on the market. These are: detergents in the form of liquids, pastes or gels, then gasoline for cleaning, ethyl alcohol, ultra-gel for cleaning, Venish. One of the Venish preparations, which is on the market, contains 5-15% bleach, less than 5% surfactant, polycarboxylates, limonene; citral; hexyl cinnamal and benzyl salicylate (Pejčić, 2016; Plavšić, 2009).

In the beginning, even at home, light petroleum derivatives, i.e. ordinary gasoline, were used as cleaning agents. As a cheap chemical, it was bought in large quantities and a person could pour gasoline into a certain container and then soak their suit in it in order to remove greasy stains. However, gasoline is very inflammable, and its vapours with air form explosive mixtures and there have been disastrous experiences. In addition, it should be stored due to elevated benzene concentrations, especially when it comes to motor gasoline. Regardless of all the above, gasoline is still used for these purposes (because it dissolves fat well), that is, it is an integral part of many cleaning products. However, it should be emphasized that it is no longer pure gasoline, but (as already said) cleaning gasoline (Pejčić, 2016; Plavšić, 2009).

Various halogen solvents are also used for this purpose. Already at the beginning of their application, it was shown that they have certain advantages in relation to gasoline. In addition to having quite satisfactory efficiency in cleaning clothes, they were neither explosive nor inflammable, which was certainly a big advantage. However, some of these agents (carbon tetrachloride, trichlorethylene) have been withdrawn from further use due to their acute and chronic toxic effects (*primarily mutagenicity and carcinogenicity*). Practically only perchlorethylene is left, which is usually mixed with some petroleum derivative (for example, gasoline). However, this compound is also classified as *causing skin irritation and skin sensitization, causing eye irritation, can cause drowsiness and unconsciousness, is suspected of causing cancer, and is toxic to aquatic life with long-term consequences* (*Sl. glasnik RS*, 2010).

Furhermore, various other inert materials or gases can be added to such preparations, when it comes to spray packages. One such agent is silica gel soaked in a mixture of organic solvents. This mixture is spread on the stain and left to dry. The organic solvent will dissolve the impurity and transfer it to the silica gel by capillary forces. When the solvent evaporates, simply remove the sand-like material with a brush, leaving a stain-free fabric underneath. Naturally, it is important how dangerous such products are for human health. Assuming that gasoline does not contain benzene and that *perchlorethylene is a Category 3 carcinogen*, various milder effects may occur. In contact with the skin, such an agent will probably *cause drying and cracking of the skin* after prolonged contact, which in turn means discomfort. Inhaling vapours in an unventilated room could cause *dizziness and drowsiness, and even fainting.* Swallowing a liquid preparation is a rather inconvenient event, but it is unlikely. *There is a burning sensation in the upper part of the digestive system, followed by nausea, which will then lead to vomiting.* The problem is not vomiting in itself, but the fact that during this reflex act, the vomited contents can be inhaled into the lungs. At the very least, the person will cough and have difficulty breathing until his condition stabilizes. In some cases*, pulmonary edema may occur, followed by difficulty breathing and suffocation*. If a child drinks a few sips of such a liquid preparation, it should immediately seek the advice of a doctor, that is, it should be taken to the nearest hospital. That is why these products must be kept out of the reach of children. In order to prevent such conditions, it would be best to take the clothes to dry cleaning and then there is nothing to fear (Plavšić, 2009; *Sl. glasnik RS*, 2010).

In addition to all the above-mentioned products, we should also *mention fabric softeners*. Housewives often use them, because our clothes smell nicer and are softer. Although they contain several substances, they are all found in extremely low concentrations. Among them all, the most present ones are those that penetrate the fiber structure and thus soften the fabric. Various aqueous suspensions of soap or oil (for example, olive oil), quaternary alkoxy ammonium salts, dimethyl-ammonium-chloride, phosphoric acid esters, fatty alcohols, anionic surfactants, and more recently chemicals containing silicon (for example , polydimethylsiloxanes). The worst of these agents are quaternary ammonium salts, anionic surfactants and silicon derivatives. As a rule, they have the following danger notices in their pure state: *it is irritating to eyes and skin*. However, their dangerous properties are lost already at concentrations below 20%. In addition, it was determined that their concentration in the water during the last wash of laundry is only 0.5%, so it is practically pointless to discuss any dangers of these agents. When more than 95% of the water is removed from the machine by centrifugation, then the remaining 5 kg of laundry contains extremely small amounts of fabric softener. It was calculated to keep them in such concentrations, i.e. so that some 2–5 µg of the agent remain per cm2 of fabric. Finally, it should be said that on the surface of the fabric, which is in contact with the child's skin, there are amounts of substances in nanograms. Those are really small amounts. Theoretically, even such small nanogram amounts *can cause irritation and redness of the skin, as well as allergic reactions*, in highly sensitive individuals. However, that is really unlikely. That is why people should not be afraid to use fabric softeners. However, it is not bad to be careful, and in the first three months of life, instead of fabric softener, you should iron the goods (Pejčić, 2016; Plavšić, 2009; *Sl. glasnik RS*, 2010).

HYGIENE MAINTENANCE IN OTHER ROOMS IN THE APARTMENT

The rooms in question are the living room, the bedroom and rooms such as the storage room or pantry, which practically represent rooms with a lower risk. Normally, no chemicals should be found in those rooms. However, some of them are occasionally used. Naturally, this does not apply to the pantry, where absolutely no chemicals are to be found. In case they must be applied (for example, due to the eradication of mold, insects, painting and varnishing), all foods, spices and additives must be removed beforehand. Therefore, due to the potential risk to the health of household members, the aforementioned products, as well as paints and varnishes, thinners, and carpet cleaners, polishers and stain removers, should not be kept in the pantries (Pejčić, 2016; Plavšić, 2009).

In order to maintain hygiene and cleanliness in the rooms, i.e. wishes that they also look clean and shiny, various chemicals are used. It is an imperative that this kind of application should take place without any possibility of causing any damage to people's health. Sometimes it is not the case, thus something should be said about this situation as well. Nowadays, furniture made of chipboard is usually bought, because it is much cheaper than furniture made of pure wood. However, while it is new, it can cause problems, as *formaldehyde is released*. The amounts of formaldehyde released are quite small, which means that they are not able (except for hypersensitive people) to cause serious adverse effects. However, it should be borne in mind that according to the Rulebook on Classification and Labeling of Substances, formaldehyde *is toxic in contact with the skin, if swallowed and inhaled, causes severe burns and eye damage, skin sensitization, and is a carcinogen* (Pejčić, 2016; Plavšić, 2009; *Sl. glasnik RS*, 2010).

In the rooms, special care must also be taken of the wood, which is used to make floors, furniture and is often found on the walls. Nowadays, such wood is already protected against various pests (for example, aphids or fungi) before being sold, so there should be no problems concerning this issue. Previously, the parquet floors were cleaned (scrubbed) with wire wool, and smeared with various means for polishing, i.e. getting a shine. Nowadays, after installation, everyone plans the parquet, and then varnishes it, which is of course a good mechanical, chemical and biological protection. For polishing parquet or furniture, chemicals are used, most often in the form of a paste, in which the main components are solid petroleum derivatives (mainly waxes) and certain gasoline-based solvents (Pejčić, 2016; Plavšić, 2009).

For better properties, manufacturers add other solvents (for example, esters or ketones for a better combination of components and a better smell), and special additives such as polymers. Most of them are inert substances, such as paraffin, but they can also contain gasoline, thus inhaling vapours can cause *drowsiness and dizziness or even a headache,* and if it gets on the skin, it causes it to dry out. *Swallowing the pasty preparation can cause nausea, and maybe even vomiting, but without other serious consequences*. That is why it is necessary for the person who cleans the parquet in this way to use gloves (for example, made of butyl rubber or PVC) and to ensure good ventilation in the room. Furthermore, these tools should definitely be kept out of the sight and reach of children (*Sl. glasnik RS*, 2010; Plavšić, 2009).

Furniture care preparations are far more demanding. They must contain almost nothing dangerous and must be made of very high-quality materials. In addition, it is useful that they also contain substances, such as disinfectants, in order to suppress bacteria or fungi on the furniture. However, among them there are some products almost similar to parquet maintenance products, but for example, instead of paraffin, they contain higher quality ingredients, such as beeswax. Disinfectants are added in quantities that cannot possibly harm human health. Nowadays, as a rule, these preparations are sold in the form of a spray, but there are also some in the form of a cream for spreading on wood. Although they are almost completely inert to human skin, it is still recommended to use PVC gloves during their application. Therefore, no harmful effects of these chemicals should be expected, and of course they should be kept out of the reach of children (Pejčić, 2016; Plavšić, 2009).

Products for the maintenance and care of flowers, and products for controlling ectoparasites in pets

Many housewives are flower lovers, so they grow various types of flowers in their apartments or terraces. In order for the flowers to look as beautiful as possible, not only the daily work around them is enough, but also various chemicals must be used for this purpose, i.e. products for plant protection, and among them most often insecticides, that is, fungicides. These agents can pose a danger to householders, not only during application, but also afterwards if (for the purpose of making the solution) they are poured into smaller containers, which are not properly washed or marked later. It should be pointed out that poisoning by these products is quite common in the house, even happening more often than in the field. Children are the most vulnerable category of all household members. According to statistics, home poisoning is most often caused by insecticides, which can also harm pets. Namely, certain insecticides are also used as antiectoparasitics in dogs, cats and birds. Poisoning in the mentioned pets can occur due to an overdose of a substance or accidental ingestion. In addition, there are other, deliberate poisonings who put certain chemicals in various baits, primarily dinitro-ortho-cresol or creosan. According to the Rulebook on the Classification, Packaging, Labeling, and Advertising of Chemicals in accordance with the Globally Harmonized System, creosan *is fatal if swallowed, in contact with the skin and if inhaled, has a corrosive effect, can cause eye damage, can cause allergic reactions to skin, causes skin irritation, is suspected of causing genetic defects, and is highly toxic to aquatic life with long-term consequences*. In addition, there is *a risk of explosion if heated indoors* (Ćupić, 2013; Plavšić, 2009; *Sl. glasnik RS*, 2010).

CONCLUSIONS

Nowadays, many chemical agents are used to maintain hygiene in homes, as well as personal hygiene.

Although these agents are potentially toxic, especially to children, most people do not think about them at all, let alone their toxic effects.

However, even in diluted form, these agents can cause irritation of the skin and mucous membranes (eyes), and the most severe forms are those that occur after ingestion.

It is believed that the most severe forms of poisoning can be caused by descaling agents, oven cleaners, bleaches and organic solvents.

If they are used in the prescribed manner, possible poisoning with these agents is really reduced to a minimum.

Buy only checked and registered products, with a proper and approved declaration (label). Preparations without a declaration or with an illegible declaration should be advertised immediately.

Never pour chemicals from their original packaging into beer, wine or similar bottles. Previous experiences show that numerous poisonings have occurred in this way (due to forgetfulness).

Always use protective equipment (apron, gloves, goggles and face mask) when applying these agents, and at least protective gloves.

Never throw the remains of dangerous chemicals or their empty packaging into the environment after use, especially into streams, rivers or lakes.

Dangerous chemicals in the house should be kept in places inaccessible to children, if possible under lock and key.

In all accidental cases, arising after exposure to a dangerous chemical, water should be applied immediately, either for rinsing or for drinking. Water will not lead to normalization of the condition in all cases, but it will certainly reduce the damage. Therefore, when working with these tools, it must always be available.

In more severe cases, medical help should be sought.

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ОРИГИНАЛНИ ЧЛАНАК

КУЋНЕ ХЕМИКАЛИЈЕ КАО МОГУЋИ УЗРОЧНИЦИ ТРОВАЊА И ЗАГАЂЕЊА ЖИВОТНЕ СРЕДИНЕ

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